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2.45-GHz microwave irradiation adversely affects reproductive function in male mouse, Mus musculus by inducing oxidative and nitrosative stress.

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Abstract

Electromagnetic radiations are reported to produce long-term and short-term biological effects, which are of great concern to human health due to increasing use of devices emitting EMR especially microwave (MW) radiation in our daily life. In view of the unavoidable use of MW emitting devices (microwaves oven, mobile phones, Wi-Fi, etc.) and their harmful effects on biological system, it was thought worthwhile to investigate the long-term effects of low-level MW irradiation on the reproductive function of male Swiss strain mice and its mechanism of action. Twelve-week-old mice were exposed to non-thermal low-level 2.45-GHz MW radiation (CW for 2 h/day for 30 days, power density = 0.029812 mW/cm(2) and SAR = 0.018 W/Kg). Sperm count and sperm viability test were done as well as vital organs were processed to study different stress parameters. Plasma was used for testosterone and testis for 3β HSD assay. Immunohistochemistry of 3β HSD and nitric oxide synthase (i-NOS) was also performed in testis. We observed that MW irradiation induced a significant decrease in sperm count and sperm viability along with the decrease in seminiferous tubule diameter and degeneration of seminiferous tubules. Reduction in testicular 3β HSD activity and plasma testosterone levels was also noted in the exposed group of mice. Increased expression of testicular i-NOS was observed in the MW-irradiated group of mice. Further, these adverse reproductive effects suggest that chronic exposure to nonionizing MW radiation may lead to infertility via free radical species-mediated pathway.

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2.45 GHz (CW) MICROWAVE IRRADIATION ALTERS CIRCADIAN ORGANIZATION, SPATIAL MEMORY, DNA STRUCTURE IN THE BRAIN CELLS AND BLOOD CELL COUNTS OF MALE MICE, MUS MUSCULUS

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Abstract—Present study examines biological effects of 2.45 GHz microwave radiation in Parkes strain mice. Forty-day-old mice were exposed to CW (continuous wave) microwave radiation (2 h/day for 30 days). Locomotor activity was recorded on running wheel for 12 days prior to microwave exposure (pre-exposure), 7 days during the first week of exposure (short-term exposure) and another 7-day spell during the last week of the 30-day exposure period (long-term exposure). Morris water maze test was performed from 17th to 22nd day of exposure. At the termination of the exposure, blood was processed for hematological parameters, brain for comet assay, epididymis for sperm count and motility and serum for SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate

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pyruvate transaminase). The results show that long-term radiation-exposed group exhibited a positive Ψ (phase angle difference) for the onset of activity with reference to lights-off timing and most of the activity occurred within the light fraction of the LD (light: dark) cycle. Microwave radiation caused an increase in erythrocyte and leukocyte counts, a significant DNA strand break in brain cells and the loss of spatial memory in mice. This report for the first time provides experimental evidence that continuous exposure to low intensity microwave radiation may have an adverse effect on the brain function by altering circadian system and rate of DNA damage.

1. INTRODUCTION

Microwaves (MW) are the electromagnetic waves with the wave length ranging from 1 mm to 1 m (frequency between 0.3 GHz to 300 GHz). These non-ionizing electromagnetic radiations have a large frequency band pattern and induce a lot of biological effects which are of great concern to human health due to their increasing use in daily life. These non ionizing radiations, generally produce heating effects, alter chemical reactions and induce electrical currents in the tissues and cells of biological system [1]. Numbers of studies have focused on RF (Radio frequency) and MW radiation-induced potential health effects and epidemiological studies have revealed that there is an increased risk of brain tumors among analogue cellular phone users [2]. In contrary, relation between exposure to RF field and cancer has not been found [3]. On the other hand, RF radiation (at 2.45 GHz and 27 MHz) is reported to increase cell proliferation [4], blood brain barrier permeability [5], Ca^{2+} efflux [6] and ornithine decarboxylase activity characteristic of many cancer promoters [7]. Protein Kinase C (PKC), a key enzyme involved in the signal transduction from membrane receptors in response to action of cytokines, growth factors, hormones etc. has also been reported to decrease in brain cells of rat exposed to RF radiation [8, 9]. These radiations are also reported to affect/ depress different neurotransmitters [10, 11] as well as structural and genomic changes in the brain and testis of rat [12]. Further, while no DNA damage in brain cells of rat has been observed after acute exposure (2 or 4 h exposure to 2450 MHz-CW) [13, 14], chronic exposure to low intensity MW (2.45 GHz and 16.5 GHz, 2 h/day/35 days) caused an increase in DNA single strand break in developing rat brain [15, 16]. Although it is well known that DNA damage in brain cells could affect neurological functions and also possibly lead to neurodegenerative disorders [17–19], it is yet to be elucidated how RF radiation may induce DNA strand break. However,

causal relation of EMF (Electro magnetic field) and biological effects such as neurological and degenerative changes can not be ruled out especially in response to chronic exposure.

A number of studies in mammals also report the effects of 2.45 GHz EMF on hematopoiesis in pregnant mice [20], spermatogenesis in mouse [21], brain development in mice [22], radial maze in rats [23], micronucleated erythrocytes in rats [24], and cholinergic activity in the rat [11]. Since susceptibility or responsiveness of various tissues and organ systems towards any stimulation or stressor including EMF exposure of different intensity and duration differs, conflicting and sometimes contradictory results are not surprising [25]. In spite of large number of studies focusing on either one or two parameters at a time, there is no comprehensive study describing the effect of EMF on various parameters of body metabolism combining behavioral responses and effects at the molecular level (DNA breakage). Moreover, there is complete lack of information on the circadian system/activity pattern following the exposure to microwave radiation. Hence present experiment was designed to study the effect of 2.45 GHz microwave radiation on various parameters, such as circadian system (locomotor activity pattern), brain function (DNA damage, spatial memory), liver function, haematological and spermatogenic parameters (sperm count, motility) of Parkes strain mice.

2. MATERIALS AND METHODS

2.1. Animals and Microwave Irradiation (Exposure Setup)

Forty day old male mice (Parkes Strain) were obtained from the mice colony of our laboratory maintained under light:dark cycle (LD12:12). Animals were supplied with food (standard rodent food pellets supplied by Pashu Aahar Kendra, Varanasi, India) and tap water *ad libitum*. Randomly selected 10 mice were divided into two groups (control and experimental) of five mice each ($N = 5$). The mice of the experimental group were exposed to 2.45 GHz microwave radiation (2 h/per day, from 09:00 h to 11:00 h for 30 days) as described below. The control group was subjected to sham exposure. The whole experiment was conducted in accordance with institutional practice and within the framework of the revised animals (Scientific procedures) act of 2002 of the Government of India on Animal welfare. Body weight was recorded weekly. Water maze test with respect to the MW exposure is presented in Fig. 1.

The experimental setup for microwave exposure is shown in Fig. 2. The microwave source consisted of Analog Signal Generator covering a frequency range from 250 kHz–20 GHz. The source (model No. E

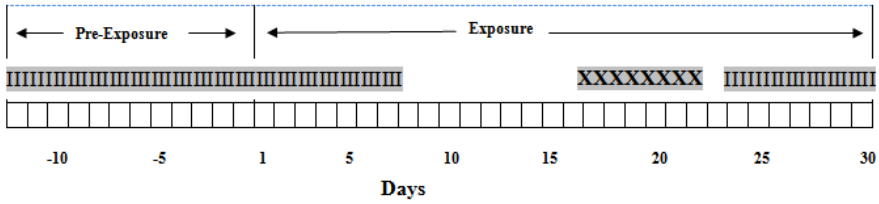


Figure 1. Diagrammatic representation of the period of monitoring of wheel running activity (IIIII) and Water maze test (XXXX) with reference to exposure to microwave radiation.

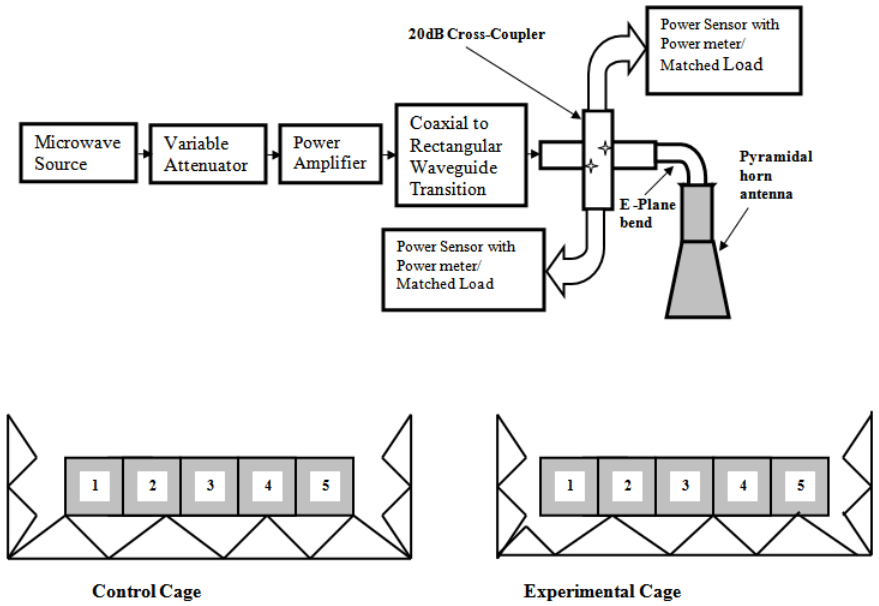


Figure 2. Diagrammatic representation of the setup for microwave radiation and the position of the animal cages during exposure.

8257D PSG) was manufactured by Agilent Technologies, USA. The required frequency was adjusted to 2.45 GHz continuous wave (CW). The microwave power was delivered to horn antenna through a coaxial attenuator, microwave amplifier (model No. 8349B, Hewlett Packard Co., USA), coaxial to waveguide transition, 20 dB cross coupler and E-plane bend. The maximum output from the amplifier was 19.8 dBm measured by the power sensor and meter having range from 30 µW to 3 W (model Nos. 8481H and 836A, Hewlett Packard Co., USA). Silver

polished brass material was used for pyramidal horn antenna. The throat dimensions of the antenna are 7.2 cm × 3.2 cm and those of horn antenna mouth are 9.0 cm × 5.0 cm. The axial length of the antenna from the throat is 10.0 cm. The distance between the pyramidal horn and the mid plane of the body of animal (assuming the body length to remain horizontal during exposure) was estimated through far field criterion ($R \geq 2D^2/\lambda$) to be 25 cm. The cross-sectional dimensions of the animal cage in E- and H-planes (electric and magnetic field planes respectively) were designed on the basis of 1.24 dB and 1.69 dB beam-widths in corresponding planes of the horn respectively. For this the horn radiation pattern were also measured at 2.45 GHz in E- and H-planes. The dimension of the animal cage was 25 cm × 16 cm × 6.5 cm accommodating five mice at a time. The cage and its partitions were made up of transparent low dielectric constant material polyethylene material (dielectric constant = 2.25). Small circular holes were drilled in the cage to keep the animals aerated during exposure and also to reduce the dielectric constant of the top surface for reducing the reflections and increasing the transmission of microwave into the cage. Each animal was kept in a pre-specified compartment of the cage throughout the exposure period. The partition was made in such a way that mice remain restrained in the cage during the period of microwave irradiation and they were exposed parallel to E field. The animal cage was placed on the carbon impregnated styrofoam microwave absorber to minimize reflection and was also tied to the absorber unit to avoid any change in the position of the cage with reference to the horn antenna. Everyday both control and experimental mice were placed in their designated cage and the compartment for two hours, i.e., from 09:00 to 11:00.

The 20 dB cross coupler, power sensor and meter having the range 30 µW to 3 W (model Nos. 8481H and 836A, Hewlett Packard Co., USA) were used to measure the power input to antenna and reflections from it. Power transmitted from the antenna was estimated by subtracting the power reflected from the antenna when it looks towards free space from the measured input power. This way power transmitted by the antenna was estimated which is equal to 54 mW. The effective relative permittivity ε_{reff} of the top surface of the perforated plastic animal cage was calculated using the following formula:

$$\varepsilon_{reff} = \frac{\varepsilon_{r1}V_1 + \varepsilon_{r2}V_2}{V_1 + V_2}$$

where ε_{r1} and ε_{r2} are the relative permittivity of the air and plastic respectively and V_1 and V_2 are their respective volumes [26]. The term 'effective' is used because the effective value of the parameters was estimated for a composite dielectric consisting air and plastic.

The values of ε_{r1} and ε_{r2} equals to 1.0 and 2.25 respectively. The expression has been used successfully in finding the effective relative permittivity of the discrete rods supporting the helix of a travelling-wave tube. For this purpose, the structure with discrete dielectric media is smoothed out as an equivalent continuous dielectric medium of an effective relative permittivity [27, 28]. The effective relative permittivity is computed to be 2.2. This was used to compute the power transmitted into the animal cage from its top surface, which is 49.54 mW. The power density was computed using the following formula:

$$Power\ Density = \frac{P_t G_t}{4\pi R^2}$$

where P_t is the power transmitted in to the cage, G_t is the gain of the horn and R is the distance between the horn aperture and mid plane of the body of exposed mice of the exposure cage and it equals to 0.02564 mW/cm^2. The SAR (specific absorption rate) was estimated for body length parallel to the electric field, as per the actual placement of mice [29]. Computed value of whole-body averaged SAR was found to be 0.03561 W/Kg.

Animals were kept in far field and exposed to very low intensity. These animals were normal through out the period of study and did not show any sign of heat stress or otherwise any discomfort. However, we have measured air temperature in the exposed and sham exposed animal cages but we did not find any difference in the temperature over the whole duration of exposure and also between the two groups.

2.2. Circadian Locomotor Activity

Both control and experimental mice were placed in individual running wheels (Clock Lab, USA) housed in a photoperiodic schedule of LD 12:12 (Light Onset at 06:00) maintained by an electronic timer (L&T; Larsen and Toubro Ltd, India). Food and water were supplied *ad libitum*. Each revolution of the wheel was taken as a count *via* a magnetic read-relay switch. Data were collected via the ACT-557 breakout box, the CL-200 data collection interface and analyzed as per the Clocklab analysis package (Coulbourn, USA) manual to generate actograms. The actograms were appropriately highlighted using Microsoft Paint software. The handling times were appropriately marked on the actograms. Individual actograms were analyzed to elucidate the phase angle difference (Ψ) with reference to the time of onset of darkness following the methods described earlier [30, 31]. The period of 3 hours consisting of exposure and handling duration was excluded to compensate for the effects of handling stress. The average phase angle difference in hours for each group was also plotted.

2.3. Morris Water Maze

Morris water maze experiment is used to test the spatial and working memory of each individual mouse. For this test each animal was placed into a small pool of water, which contains an escape platform hidden a few millimeters below the water surface. The mice were released in the four different quadrants sequentially for checking the escape latency time (ELT). Three different types of tests include: visible platform training (1 day), hidden platform test (4 days) and the probe test (without platform, 1 day). The timings (ELT) obtained for both groups were compared. This whole experiment was continued for 6 days, including last day for the probe test [32].

All the mice were taken to the experimental Morris water maze pool. The mice were allowed for 20 s to locate the platform in a target quadrant (Q4), and then were allowed to stay on the platform for 20 s. If the mice were unable to locate the platform within the time then they were guided by hand to the platform and were allowed to remain there for 120 s. Every time, the reaching time to the platform (ELT) was noted. After four-day of the hidden platform test, on the last day the platform was removed and the time spent by each animal in the target quadrant was noted. Starting position of mice on each day to conduct four acquisition trials were changed regularly.

2.4. Hematology

After the microwave exposure of 30 days, blood was collected from the tail vein to determine the number of erythrocytes & leucocytes by hemocytometric method and hemoglobin by hemometer (Sahli's method).

2.5. Spermatogenic Count

The mice were sacrificed by decapitation. Body was dissected; the epididymis was excised and placed in a pre-warmed Petri dish containing 0.5 ml of 0.9% NaCl at 37°C. The tissue was minced with scalpel for approx. 1 min and then placed in a 37°C incubator for 10 min prior to determining sperm motility. The suspension was stirred, one drop was placed on a warmed microscope slide, and a 22×60 mm cover slip was added on the top. For the determination of sperm motility, at least 10 microscopic fields were observed at $40\times$ magnification using a standard optical microscope, and the percentage of motile sperm was determined [33]. Thereafter, the cover slip was removed and the spermatozoa suspension was allowed to dry in air. The dried sample was stained with 1% eosinY/5% nigrosin and

examined at 40× for assessing sperm counts according to the WHO laboratory manual [34, 35].

2.6. Comet Assay

For the Comet assay experiment brain was taken out immediately from the mice sacrificed as described above and processed for the preparation of a single-cell suspension according to the method of Hartmann et al. [36] as described by Patel et al. [37]. Briefly, brain was placed in 1 ml chilled mincing solution (Hank's balanced salt solution, with 20 mM EDTA and 10% DMSO) in a Petri dish and chopped into small pieces with a pair of scissors. The pieces were allowed to settle and the supernatant containing the single cells was taken. Normal melting agarose (NMA, 1.0%) was prepared in Milli-Q water, microwaved and kept at 60°C. Conventional glass slides were then dipped into molten NMA up to two-thirds of their frosted end, the lower surface was wiped clean and the slides were left to dry. On this first layer, 80 µl of diluted sample (100 µl cell suspension mixed with 100 µl of 1% low melting point agarose, LMA) was added to form the second layer. A cover slip (size 24 mm × 60 mm) was placed gently to evenly spread the cells in the agarose. The slides were kept on ice for 5 min to allow the gel to solidify. The cover slips were removed and a third layer of 0.5% LMA (90 µl) was added onto the slide and placed over ice for 10 min. Finally, the cover slips were removed and the slides immersed in freshly prepared chilled lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10) with 10% DMSO and 1% Triton X-100 being added just before use. The slides remained in the lysing solution over night at 4°C, followed by electrophoresis according to the method of Singh et al. [38]. The slides were placed in a horizontal gel electrophoresis tank side-by-side and avoiding spaces, with agarose ends nearest to the anode. Fresh and chilled electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) was poured into the tank to a level of approximately 2.5 mm above the slides. The slides were left in this solution for 25 min to allow DNA unwinding and expression of alkali-labile sites as DNA strand breaks. Electrophoresis was conducted at 24 V (0.7 V/cm) and a current of 330 mA using a power supply (Electra Comet III from Techno Source India Pvt. Ltd., Mumbai, India) for 30 min at 4°C. All these steps were performed under dimmed light and the electrophoresis tank was covered with black paper to avoid additional DNA damage due to stray light. After electrophoresis, the slides were drained and placed horizontally in a tray. Tris buffer (0.4 M; pH 7.5) was added drop-wise and left for 5 min to neutralize excess alkali. Neutralizing of slides was repeated thrice. Each slide was stained with 75 µl EtBr (20 µg/ml) for 5 min and dipped in chilled distilled water to wash off

excess EtBr, and cover slip placed over it. Slides were placed in a dark humidified chamber to prevent drying of the gel. The slides were scored within 24 h.

Slides were scored using an image-analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (Leica, Germany) equipped with appropriate filters (N2.1, excitation wavelength of 515–560 nm and emission wavelength of 590 nm). The microscope was connected to a computer through a charge-coupled device (CCD) camera to transport images to software (Komet 5.0) for analysis. The final magnification was $\times 400$. The Comet parameters recorded were Olive tail moment (OTM, arbitrary units), tail DNA (%) and tail length (migration of the DNA from the nucleus, μm). Images from 100 cells (50 from each replicate slide) were analyzed as per the *in vivo* guidelines [36].

2.7. SGOT and SGPT Test

Trunk blood of the decapitated mice was collected in a tube and centrifuged at 4000 rpm for 15 min. Serum was separated and stored in -20°C until assayed. The level of SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate pyruvate transaminase) in the serum was measured with the help of SGOT-SGPT kits as per manufacturer's protocol (Span Diagnostics Ltd., India).

2.8. Statistical Analysis

The means of the control and experimental groups were compared using Student's 't' test. The null hypotheses were rejected at $p < 0.05$.

3. RESULTS

There was no significant difference in the body weight of the MW-exposed mice as compared to control or its initial weight.

3.1. Circadian Locomotor (Wheel Running) Activity

All mice exhibited positive phase angle differences (Ψ) between lights-off and the onset of activity. A statistically significant difference was witnessed when average Ψ of the long term (30 days) microwave-exposed group was compared with that of its time qualified control group and all other groups. The average Ψ of the short term (7 days) microwave-exposed group was not significantly different from that of its time-qualified control and pre-exposed control groups (Fig. 3).

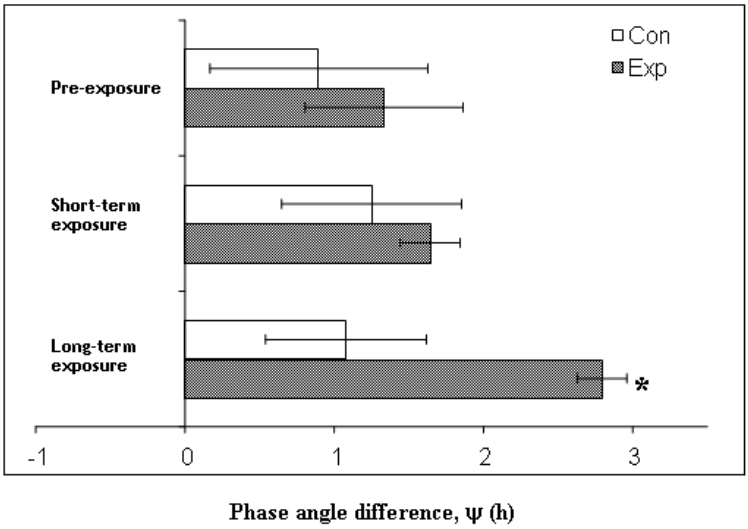


Figure 3. Phase angle difference (Ψ) in hours (h) between lights-off and the onset of activity of mice. $^{*}p < 0.05$, significance of difference from control group.

There was a marked shift in the activity bouts after long-term exposure to radiation, reflecting most of the activity occurring within the light fraction of the LD cycle. In contrast, in pre-exposure groups and to some extent in short-term exposed groups, most of the activity was restricted to the dark phase (Fig. 4).

Note marked shift in the activity bouts after long-term exposure to radiation (mostly occurring within the light phase of the LD cycle) in comparison to its pre-exposure and short term exposure period. Most of the activity is restricted to the dark phase in the sham exposed control group.

3.2. Morris Water Maze

In the Morris water maze experiment there was no significant difference among the ELT of control and exposed mice during hidden platform test. However, the mice exposed to MW radiation spent significantly less time in the Q-4 during probe test (6th day) as compared to control (Fig. 5).

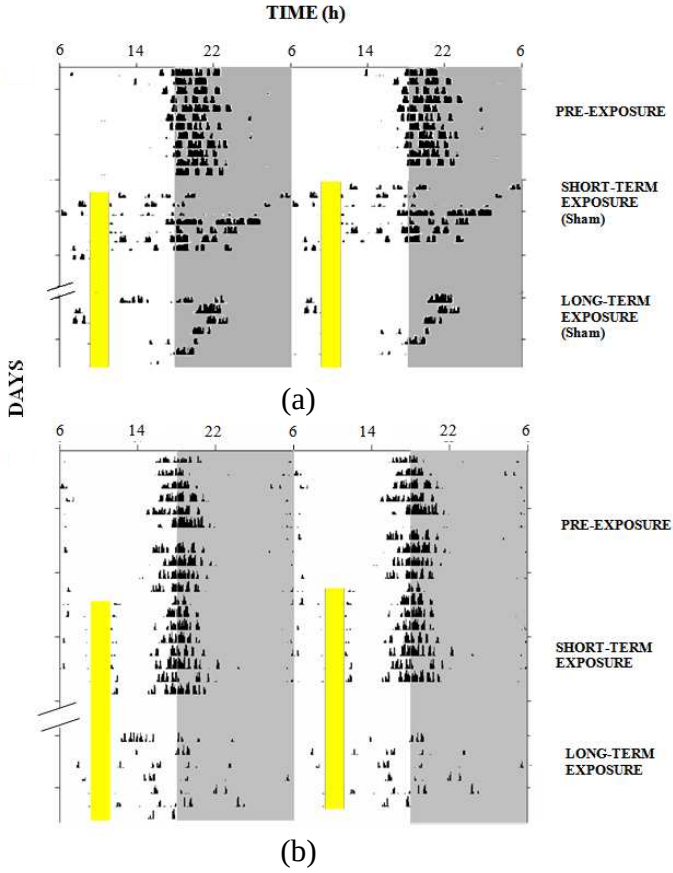
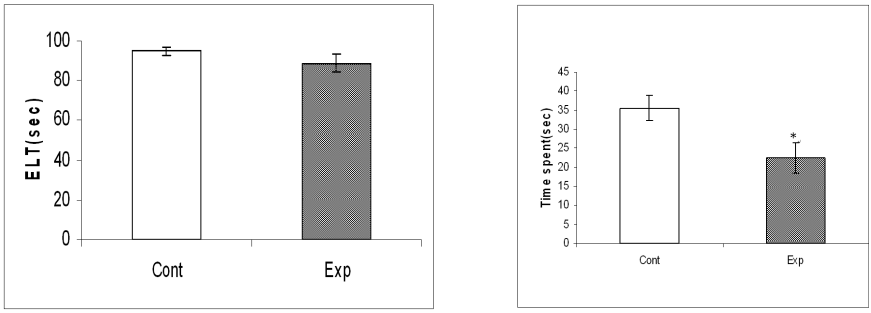


Figure 4. Representative actograms showing wheel running activity of control (upper panel; (a)) and microwave radiation exposed (lower panel; (b)) mice maintained under LD 12:12. Vertical bars represent period of microwave exposure (09:00–11:00).

3.3. Hematology

After the long-term MW radiation exposure, although there was no significant change in the hemoglobin content, the total number of erythrocytes and leucocytes increased significantly in the experimental mice as compared to the control (Fig. 6).



(a) (b)

Figure 5. Escape latency time (ELT) of mice during water maze test to locate the hidden platform; (a) and the time spent in Q-4 (target quadrant) during last day when platform was removed, i.e., probe test; (b) $^{*}p < 0.05$, significance of difference from control.

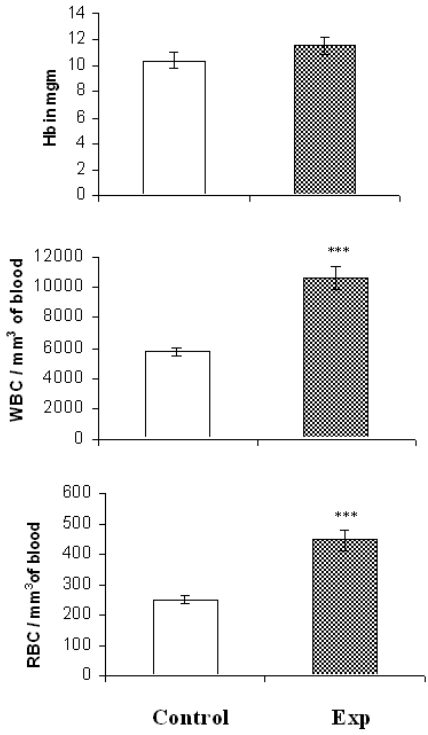
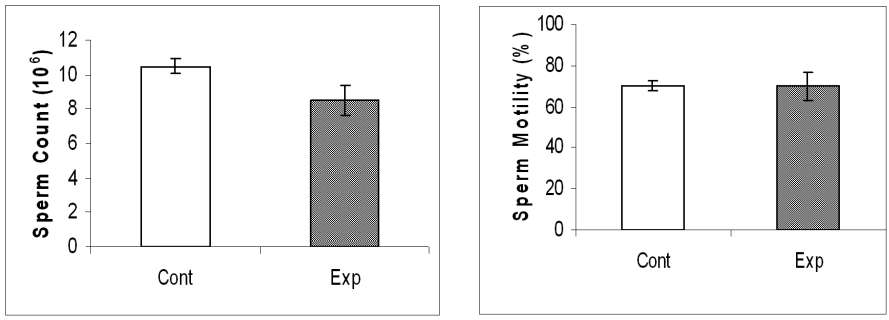


Figure 6. Effect of 2.45 GHz microwave radiation on the hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) counts of mice. $^{***}p < 0.001$ significance of difference from control.

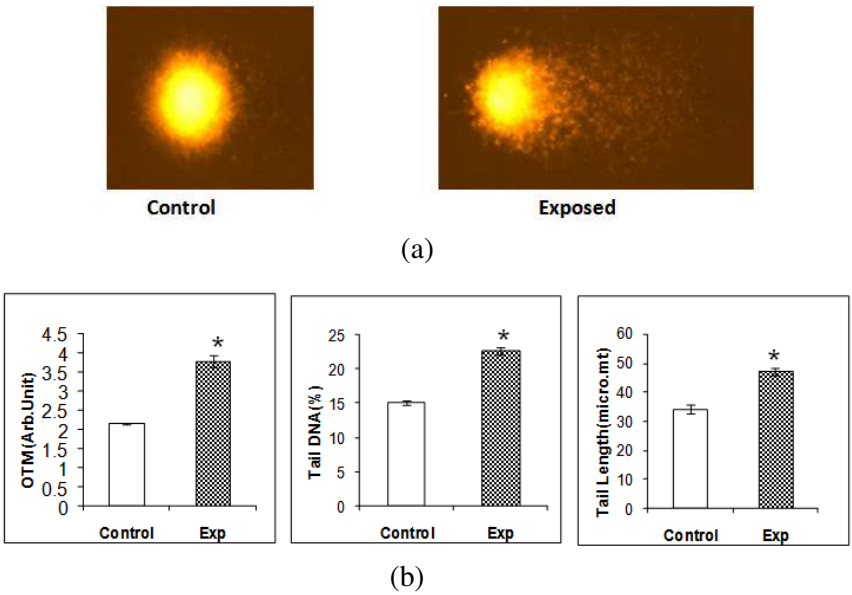
3.4. Sperm Counts

The sperm counts and the % of sperm motility were not altered in the treated mice as compared to the control (Fig. 7).



(a) (b)

Figure 7. Effect of 2.45 GHz microwave radiation on the sperm count (a) and sperm motility (b) of mice.



(a)

(b)

Figure 8. Effect of 2.45 GHz radiation on the DNA damage of brain cells in mice monitored by Comet assay.

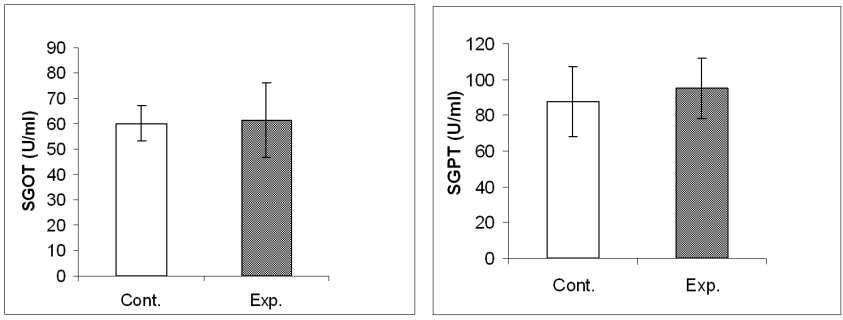
Upper panel of the Fig. 8(a) shows the photographs of intact (control) and damaged DNA (exposed) following long term exposure. In the comet assay image the magnification is 400×. Note significant increase in the tail length following DNA breakage in the exposed group. Lower panel (b) shows quantitative measurement of olive tail moment, % tail DNA and tail length. $^*p < 0.05$, significance of difference from control.

3.5. Comet Assay

A significant ($p < 0.05$) increase in the Olive tail moment (OTM), tail length and % tail DNA were observed in brain cells of microwave-exposed mice when compared to control brain cells (Fig. 8).

3.6. SGOT and SGPT Test

The levels of SGOT and SGPT are used as blood based markers of liver damage. There were no significant differences in the levels of SGOT and SGPT in the exposed mice as compared to the control (Fig. 9).



(a) (b)

Figure 9. Effect of 2.45 GHz microwave radiation on the serum glutamate pyruvate transaminase (SGOT; (a)) and serum glutamate pyruvate transaminase (SGPT; (b)) of mice.

4. DISCUSSION

In general, although the chronic exposure (2 h/day for 30 days) to 2.45 GHz microwave (CW) had no effect on the general body metabolism, it affected the circadian organization and spatial memory of mice. The long-term exposure to this non-ionizing radiation also led to strand DNA breakage in the brain cells as judged by the comet assay.

Monitoring the wheel running activity during pre-exposure, shot-term exposure and long-term exposure period clearly indicates that chronic exposure to 2.45 GHz MW radiation alters the characteristics of the circadian rhythms in locomotor activity of mice. From the actograms, it is apparent that under the long-term, but not short-term exposure regime, the activity tends to be suppressed in intensity. The large positive Ψ (advance of onset) exhibited by the animals exposed to radiation for longer durations could be due to the exposure of MW radiation early in the subjective day. Moreover, the activity seems to fragment into several components and shows a tendency towards splitting, even in the LD condition. It also appears that, long-term exposure to MW radiation caused a suppression of activity and reduced entrainment response to the imposed LD schedule. It may be surmised that the radiation is a weaker Zeitgeber than light, albeit it could be an effective one.

Further, these animals exhibit a bout of post-replacement activity, after wheel deprivation during the handling period, as reported earlier [39]. However, all the experimental animals tend to continue running after the post-handling bout, whereas control animals show a clear separation between the post-handling bout and the onset of activity. Further studies, especially in DD (constant darkness) or LL (constant light) are required to investigate the possible circadian-rhythm modifying effects of this radiation and to elucidate a phase-response curve. It may be tentatively stated that, the rest-activity ratios may be altered by this radiation. Moreover, the duration of long-term exposure could be increased further, in expectation of a concomitant increase in the phenomena discussed above. Present rhythm study indicates that microwave radiation has chiefly deleterious effects on the circadian physiology of mammals, evinced by suppression and disorganization of locomotor activity rhythm, shifting of the onset of locomotor activity, and failure to entrain to an LD cycle, not with short-term exposure, but when the exposure is relatively chronic.

The water maze test developed by Morris [32] is a behavioral procedure designed to test spatial memory in rodents. The spatial memory is part of memory responsible for recording information about one's environment. It helps in gathering and processing sensory information about its surroundings. In mammals the spatial memory is hippocampus dependent. The Morris water maze has proven to be a robust and reliable test that is strongly correlated with hippocampal synaptic plasticity and NMDA (N-methyl D-aspartate) receptor function. Present study shows that the learning increases continuously with the trials in both control and the exposed mice since they show improvement in their performance in locating the

hidden platform and ELT start decreasing suggesting that non-ionising MW radiation has no obvious adverse effect on the learning process. However, significant difference was seen in the probe test. The radiation exposed mice spent significantly less time in the target quadrant as compared to control. This preliminary study although suggests that spatial memory may be affected by such exposure, further studies especially the effect of short- and long-term exposures on memory, are required to strengthen this suggestion.

Microwave radiation increased the number of erythrocytes and leucocytes in irradiated mice, a condition similar to that observed in polycythemia (increased number of RBC) and leukemia (increased number of WBC) but there was no significant change in hemoglobin content. In rats an increase in leucocytes number but no difference in differential leucocytes count after four weeks of exposure at 100 Hz magnetic field has been reported [40]. Leucocytes are much more sensitive than erythrocytes. Black and Heynick [41] reported that exposure to radiofrequency electromagnetic fields can stimulate lymphocytes to become lymphoblasts (active cells undergoing mitosis). Although present findings indicate a significant increase in blood cell counts following microwave radiations, mechanism of such increase and its pathology needs to be confirmed before drawing meaningful conclusion.

Our data obtained from alkaline comet assay illustrated that prolonged exposure to microwave radiation causes DNA strand breaks in brain cells of mice. The data is consistent with a study by Paulraj and Behari [16], which demonstrated that thirty five days prolonged exposure to low intensity microwave can cause DNA strand break in the brain cells of rat. Another study reported that even acute low intensity microwave exposure (2 h) can produce a significant increase in the DNA strand breaks in rat brain [17]. In spite of number of reports indicating radiation induced DNA damage, the precise mechanism of DNA strand breaks due to microwave radiation is still unknown. However, there may be two possible pathways of DNA strand break: (i) an induction in DNA damage could be due to a reduction in the DNA damage repair processes in the cells or due to DNA–DNA and DNA–protein cross links and DNA adduct formation etc. which are generated as an intermediate step, during DNA repair; (ii) microwave radiation creates own magnetic field which can initiate Fenton reaction that generate free radical in brain cells leading to DNA strand breaks [9, 15, 17]. DNA strand break even after 2 hours of exposure indicates extreme sensitivity of brain cells to EMF, suggesting its adverse effect on brain function.

5. CONCLUSION

Based on the present findings, it is concluded that chronic exposure to microwave radiation alters blood picture and has degenerative effects on brain performance gauged from changes in spatial memory, circadian organization and extent of DNA damage. Although MW radiation exposure has no adverse effect on the reproductive and metabolic performance, prolonged exposure may lead to neurodegenerative disorder. Since brain performance is directly correlated to human health risk assessment; similar to ionizing radiation, safe limits to the frequency and duration of microwave radiation must be also prescribed in view of its increased use in the daily life of the modern society.

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Cognitive impairment and neurogenotoxic effects in rats exposed to low-intensity microwave radiation.

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Abstract

The health hazard of microwave radiation (MWR) has become a recent subject of interest as a result of the enormous increase in mobile phone usage. The present study aimed to investigate the effects of chronic low-intensity microwave exposure on cognitive function, heat shock protein 70 (HSP70), and DNA damage in rat brain. Experiments were performed on male Fischer rats exposed to MWR for 180 days at 3 different frequencies, namely, 900, 1800 MHz, and 2450 MHz. Animals were divided into 4 groups: group I: sham exposed; group II: exposed to MWR at 900 MHz, specific absorption rate (SAR) 5.953 × 10(-4) W/kg; group III: exposed to 1800 MHz, SAR 5.835 × 10(-4) W/kg; and group IV: exposed to 2450 MHz, SAR 6.672 × 10(-4) W/kg. All the rats were tested for cognitive function at the end of the exposure period and were subsequently sacrificed to collect brain. Level of HSP70 was estimated by enzyme-linked immunotarget assay and DNA damage was assessed using alkaline comet assay in all the groups. The results showed declined cognitive function, elevated HSP70 level, and DNA damage in the brain of microwave-exposed animals. The results indicated that, chronic low-intensity microwave exposure in the frequency range of 900 to 2450 MHz may cause hazardous effects on the brain.

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Confirmation studies of Soviet research on immunological effects of microwaves: Russian immunology results.

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Abstract

This paper presents the results of a replication study performed to investigate earlier Soviet studies conducted between 1974 and 1991 that showed immunological and reproductive effects of long-term low-level exposure of rats to radiofrequency (RF) electromagnetic fields. The early studies were used, in part, for developing exposure standards for the USSR population and thus it was necessary to confirm the Russian findings. In the present study, the conditions of RF exposure were made as similar as possible to those in the earlier experiments: Wistar rats were exposed in the far field to 2450 MHz continuous wave RF fields with an incident power density in the cages of 5 W/m² for 7 h/day, 5 days/week for a total of 30 days, resulting in a whole-body SAR of 0.16 W/kg. Effects of the exposure on immunological parameters in the brain and liver of rats were evaluated using the complement fixation test (CFT), as in the original studies, and an additional test, the more modern ELISA test. Our results, using CFT and ELISA, partly confirmed the findings of the early studies and indicated possible effects from non-thermal RF exposure on autoimmune processes. The RF exposure resulted in minor increases in formation of antibodies in brain tissue extract and the exposure did not appear to be pathological. In addition, a study was conducted to replicate a previous Soviet study on effects from the injection of blood serum from RF-exposed rats on pregnancy and foetal and offspring development of rats, using a similar animal model and protocol. Our results showed the same general trends as the earlier study, suggesting possible adverse effects of the blood serum from exposed rats on pregnancy and foetal development of intact rats, however, application of these results in developing exposure standards is limited.

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Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats

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Go to:

Abstract

Background:

Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time.

Objective:

The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats.

Materials and Methods:

Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835×10^{-4} W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672×10^{-4} W/kg. At the end of the

exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay.

Results:

In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain.

Conclusion:

We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

Keywords: Brain, comet assay, deoxyribonucleic acid damage, microwave radiation

Go to:

INTRODUCTION

The electromagnetic field generated from the extremely low frequencies (30-300 Hz) to radiofrequency and microwaves (100 kHz-300 GHz) is usually recognized as non-ionizing radiation. Microwave radiation is a part of non-ionizing electromagnetic radiation present in the environment and this radiation has become a threat to human health since the introduction of wireless communication system and its increased usage.[1,2] The environmental issues surrounding mobile phones are proving to be a sizeable challenge. The potential adverse effects of these non-ionizing radiations may depend on their accumulation over a long period of time. Public concerns about possible hazardous effects of exposure to these frequencies are increasing in our society due to increased usage of devices such as mobile phones, TV, Wi-Fi, etc.

Effect of microwave radiation exposure on induction of deoxyribonucleic acid (DNA) damage is a subject of keen interest. Studies reporting *in vivo* and *in vitro* genotoxic effects of microwave radiation exposure are contradictory and intriguing.[3,4] Vijayalaxmi *et al*.,[5] have reported no evidence for induction of DNA single-strand breaks in human blood lymphocytes exposed *in vitro* to pulsed-wave 2450 MHz radiofrequency radiation for 4 h. McNamee *et al*.,[6] have not found evidence of genotoxicity in human blood cell cultures after 24 h and exposure of 1.9 GHz. In addition, some studies using experimental animals have also given contradictory reports on genotoxic effects of microwave radiation.[7,8]

Although, there are some studies that have reported DNA damaging effect of microwave exposure at high level, i.e., up to 1-2 W/kg of specific absorption rate (SAR), but to the best of our knowledge, there is no study until date reporting the effect of microwave radiation exposure at very low-level with various frequencies. In the present study, we tried to bridge this gap by using three frequencies at very low-level of microwave exposure in male Fischer rats. The Comet assay is a sensitive technique which has widely been used to detect biological effects of various environmental or occupational substances on DNA in humans and animals.[9,10,11,12] Hence, this technique is highly useful in evaluating the extent of DNA damage at primary level even after a duration of 30 days exposure to microwave radiation.

In view of the above, the present study was designed to assess the possible DNA damaging effects of chronic and low level microwave radiation exposure at different frequencies *viz*. 900, 1800 and 2450 MHz in brain tissue of male Fischer rats. For this purpose, the highly sensitive alkaline comet assay method was used to detect primary DNA damage.

Go to:

MATERIALS AND METHODS

Microwave exposure setup and dosimetry

The gigahertz transverse electromagnetic (GTEM) cell, GTE10 is used for the microwave exposure and has been designed with the help of Center for Applied Research in Electronics (Microwave Laboratory), Indian Institute of Technology, New Delhi and Amitech Electronics Ltd. Sahibabad, Ghaziabad (U.P) to estimate biological effects of microwave radiation [Figure [Figure1a1a and andb].b]. GTEM cell is a pyramidal tapered, dual terminated section with its outer cell dimension as L: 220 cm × B: 120 cm × H: 80 cm. Microwaves are generated from microwave generator SMC 100 (Rohde and Schwarz GmbH and Co, Germany). The microwave source consists of a signal generator operating at frequency range from 9 KHz to 3.2 GHz, an amplifier, a direct current DC regulator and a power meter. During the exposure rats were restrained in a L: 30 cm × B: 15 cm × H: 20 cm closed box divided into four compartments with holes of 1 cm diameter to facilitate easy movement and breathing, kept at a distance of 100 cm from source. One box can hold four rats and two such boxes can be placed within the GTEM cell, thus, 8 rats can be exposed at a time in GTEM cell. The microwave chamber is lined with absorbers which minimize the possibility of any reflections. Electric field has been experimentally checked by using an E-field probe inserted into the transverse electromagnetic (TEM) cell through a slit wall. The GTEM cell was placed in a temperature controlled room under constant lighting conditions. SAR distribution was calculated by Power Balance Method using the equation:[13]

P_{abs} per mouse = 1/n (P_{in} − P_{out} − P_{refl})

Where, P_{abs} = radiofrequency RF power in watt absorbed per animal, *n* = number of animals within the cell, P_{in} = input power (Watt), P_{out} = output power (Watt) and P_{refl} = reflected power (Watt).

Animal exposure

Male Fischer-344 rats weighing 150-200 g were obtained from central animal house facility of the institute and placed in individual raised, galvanized wired cages, kept under standard conditions (temperature 22 ± 2°C) under alternating 12 h light and dark cycle. They were provided with nutritionally adequate standard diet obtained from Nutrilab (Bangalore, India) and water *ad libitum*. Animals were divided into four groups (6 animals in each group): Group I (sham exposed): Animals kept under same conditions as that of other groups except microwave exposure, Group II: Animals exposed to microwave radiation at 900 MHz, SAR 5.953 × 10^{-4} W/kg, Group III: Animals exposed to 1800 MHz, SAR 5.835 × 10^{-4} W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 10^{-4} W/kg. Animals were exposed to microwave radiation in a TEM cell (Amitech Electronics Ltd, India) at above mentioned frequencies for 2 h/day, 5 days/week (power level 0.00 dBm) during light period and every day at the same time for 30 days. The sham exposed group was subjected to similar conditions except the microwave exposure. Appropriate permission was taken from Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi and care of the animals was undertaken as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals India for laboratory animal facilities.

Deoxyribonucleic acid damage analysis using alkaline comet assay

DNA damage was evaluated using the alkaline comet assay with some minor modifications.[14,15,16] Slides were prepared in duplicates per sample. For the comet assay experiment brain was taken out immediately from the sacrificed rats and washed with cold phosphate buffer saline (PBS). Briefly, brain was placed in 1 ml chilled mincing solution (Hank's balanced salt solution, with 20 mM Ethylene diamine tetra acetic acid EDTA and 10% dimethyl sulfoxide DMSO) in a petri dish and chopped into small pieces with a pair of scissors to get a uniform cell suspension. Slides were pre-coated with 600 µl of low melting agarose (LMA, 1.0%) prepared in PBS. On this first layer, 600 µl of diluted sample (50 µl cell suspension mixed with 600 µl of 0.75% LMA) was added to form the second layer. The slides were kept on ice for 5 min to allow the gel to solidify. The slides were immersed in freshly prepared chilled lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10) with 10% DMSO and 1% Triton X-100 added just before use. The slides remained in the lysing solution for 1 h at 4°C, followed by electrophoresis in a horizontal gel electrophoresis tank with agarose ends nearest to the anode. Fresh and chilled electrophoresis buffer (1 mM Na_2 EDTA and 300 mM NaOH, pH > 13)

was poured into the tank up to a level of approximately 2.5 mm above the slides. The slides were left in this solution for 25 min to allow DNA unwinding and expression of alkali-labile sites as DNA strand breaks. Electrophoresis was conducted at 0.9 V/cm for 60 min at 4°C. All these steps were performed under dim light and the electrophoresis tank was covered with black paper to avoid additional DNA damage due to stray light. After electrophoresis, the slides were drained and placed horizontally in a tray. Tris buffer (0.4 M; pH 7.5) was added drop-wise and left for 5 min to neutralize excess alkali. Neutralization of slides was repeated three times and subsequently slides were dried and stored.

Dried slides were rehydrated and each slide was stained next day with 100 µl of Ethidium Bromide (20 mg/ml) for 5 min. Slides were randomized and coded to blind the scorer. All slides were scored by one person to avoid inter-scorer variability. Slides were scored using an image-analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (BX 51, Olympus Japan). The microscope was connected to a computer through a charge-coupled device camera to transport images to software (Komet 5.0) for analysis. Images from 100 cells (50 from each replicate slide) were analyzed. Undamaged cells had an intact nucleus without a tail and damaged cells had the appearance of a comet. To quantify DNA damage, following parameters were evaluated: Percent of DNA content in head and tail, Olive tail moment (OTM) and tail length (TL) using Komet 5.0 software (Kinetic Imaging, Liverpool, UK) as described by Tice *et al*.[17]

Statistical analysis

All values were expressed as mean ± standard error. Statistical analysis was performed with SPSS (version 16.0). Significance of differences among groups was determined by one way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was accepted at P value < 0.05.

Go to:

RESULTS AND DISCUSSION

The results of the present study indicate increase in single strand DNA breaks in brain tissue of rats chronically exposed to microwave radiation. In comparison with control the comet assay performed in brain tissue established a significant increase in the percent of DNA in tail, OTM and TL [Figures [Figures22–4]. The percent of DNA migrating in to tail region was significantly enhanced ($P < 0.001$) in all the three groups i.e., 900 MHz, 1800 MHz and 2450 MHz. Correspondingly, the percentage of DNA in head was significantly decreased in all the microwave exposed groups. The % of DNA in head and tail of group exposed at 2450 MHz was found significantly different from the 900 MHz exposed group [Figure 2]. The OTM also increased significantly ($P < 0.05$) in the group of animals exposed to 900 MHz as well as in animals exposed to 1800 and 2450 MHz [$P < 0.01$; Figure 3]. The TL of comet was also increased significantly ($P < 0.05$) in animals exposed to 900 and 1800 MHz as well as in the animals exposed to 2450 MHz [$P < 0.001$; Figure 4]. However, there was no significant difference in the body weight of the rats exposed to microwave radiation as compared to controls or its initial weight.

The present study aimed to find out the possible effects of chronic low-level microwave exposure on DNA damage in brain tissue of experimental rats. This study provides evidence of DNA damage after 30 days of exposure to rats with three different frequencies of microwaves, i.e., 900 MHz, 1800 MHz and 2450 MHz and at a whole body SAR value of 5.953×10^{-4} W/kg, 5.835×10^{-4} W/kg, and 6.672×10^{-4} W/kg respectively in a GTEM Cell. An earlier study has reported that the low intensity electromagnetic field is capable of interacting with cellular processes associated with carcinogenesis.[18] The integrity of genetic material is a prerequisite for the well-being of living system and its damage is closely related to the pathophysiological condition of cells. Investigation of the biological effects of radiofrequency electromagnetic field requires the assessment of direct and indirect effects on genomic DNA and is one of the most active areas in this field. Cells are unusually sensitive to electromagnetic fields and are the primary targets of radiation.[19,20] The weak radiofrequency electromagnetic field may accelerate electron transfer and thereby destabilize the cellular macromolecules.[21,22] However, the weak electromagnetic fields are not sufficient directly to break a chemical bond in DNA. Therefore it can be concluded, that genotoxic effects are mediated by indirect mechanisms.

The comet assay also called Single Cell Gel Electrophoresis assay is a very sensitive and established genotoxicity assay for the estimation of DNA damage at the individual cell level *in vivo* and *in vitro*. The damage usually appears as single stranded and/or double stranded breaks in DNA. It is also used to measure the presence of different types of DNA altering lesions. The alkaline comet assay used in this study is based on the alkaline lysis of labile DNA at sites of damage.[23] The results of the present study established that prolonged exposure of microwave radiation to rats causes DNA strand breaks in brain cells. Paulraj and Behari[24] reported single strand DNA breaks in Wistar rat brain exposed to low intensity microwave radiation exposure for 35 days (2.45 and 16.5 GHz, SAR 1.0 and 2.01 W/kg respectively). However, the present study shows similar effects at even lower frequencies of microwave exposure in Fischer rats.

The present study demonstrates significant ($P < 0.001$) increase in percentage of DNA in tail and tail moments in all groups exposed to microwave radiation. The SAR values in this study are far below the limit of 2 W/Kg for possible exposure to head in human as per the International Commission on Non-Ionizing Radiation Protection guidelines.[25] Significant percentage of tail DNA in microwave exposed animals indicates that these radiations may act as genotoxic agents. Our earlier findings suggest that microwave exposure at low-levels causes alteration in cognitive function and heat shock protein levels in rat brain.[26] Our another study also suggests that there is a role of microwaves in inflammation and oxidative stress.[27] The results of present study are in accordance with some earlier studies suggesting that DNA damage is indeed caused by microwave exposure in humans.[22,28,29,30] Reports obtained so far on microwave radiation induced DNA damage are controversial and intriguing, and the precise mechanism of DNA strand breaks due to microwave radiation still remains unknown. However, DNA strand breaks may occur due to residual damage resulting from impairment of DNA repair or due to DNA-DNA and DNA-protein cross links and/or DNA adduct formation. Perhaps, the magnetic field created by microwave radiation could lead to generation of free radicals in the brain cells that may induce DNA strand breaks.[8,31,32] The magnetic field components of an electromagnetic field can delay the recombination rate of free radical pairs which causes radical to stay free longer and gives them more potential to do more damage.

The results of the present study showed a significant OTM due to low level microwave exposure in the group exposed to 900 MHz ($P < 0.05$) as well as 1800 and 2450 MHz ($P < 0.01$) [Figure 4]. The concept of tail moment as a metric for DNA migration was introduced by Olive *et al*.[33] and called as OTM. Tail moment is the product of the TL and the fraction of total DNA in the tail. The increase in the OTM may be due to indirect induction of DNA strand breaks and/or generation of such modifications in DNA, which can be transformed into DNA strand breaks, because it is obvious that intrinsic quantum energy (E = hu) of microwaves is too low to dislodge an electron from macromolecules.

In this study, we observed significant migration in length of tail DNA in groups exposed to 900 ($P < 0.05$), 1800 ($P < 0.01$) MHz and in group exposed to 2450 MHz ($P < 0.001$). It has been reported that exposure to 1800 MHz, SAR 1.2 W/kg induces DNA strand breaks in human fibroblasts and rat granulosa cells.[33] The results of the present study are in accordance with earlier studies, where it has been reported that chronic exposure to microwave radiation for 35 days at 2.45 and 16.5 GHz, SAR 1.0 and 2.01 W/kg respectively, causes single strand DNA breaks in Wistar rat brain cells.[24,34] DNA strand breaks, if not properly repaired, are known to lead cell death. Since nerve cells do not divide and the damage accumulates, the more likely consequences of DNA damage in nerve cells may be cell death, which could either lead to or accelerate the development of neurodegenerative diseases.[32] Factors that can influence DNA strand damage in a particular tissue include cell type heterogeneity, cell turnover frequency, cell cycle etc., Different cell types may have different background levels of DNA strand breaks due to variation in its metabolic activities.[35] Through a homeostatic mechanism, cells maintain a delicate balance between spontaneous and induced DNA damage. DNA damage accumulates if such a balance is altered, which may in turn affect cell functions.

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CONCLUSION

Based on the present findings, it is concluded that chronic microwave radiation exposure at low-level induces DNA damage. Prolonged exposure may lead to neurodegenerative disorders. Although, the

energy of microwaves is not sufficient directly to break a chemical bond in DNA, genotoxic effects may be mediated by indirect mechanisms such as generation of oxygen free radicals or a disturbance in DNA-repair processes. In order to minimize the exposure, safe limits with respect to frequency and duration of microwave radiation must also be prescribed in view of its increased use in society. However, knowledge of the possible health effects of microwave radiation is still inadequate and inconclusive. Though, the present study seems preliminary, this study is providing the evidence of the effect of microwave radiation at very low-level of exposure and at the lower, middle and upper frequency used in mobile telecommunication. Further exploration is needed to reach any concrete conclusion.

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Go to:

Footnotes

Source of Support: ICMR, New Delhi.

Conflict of Interest: None declared.

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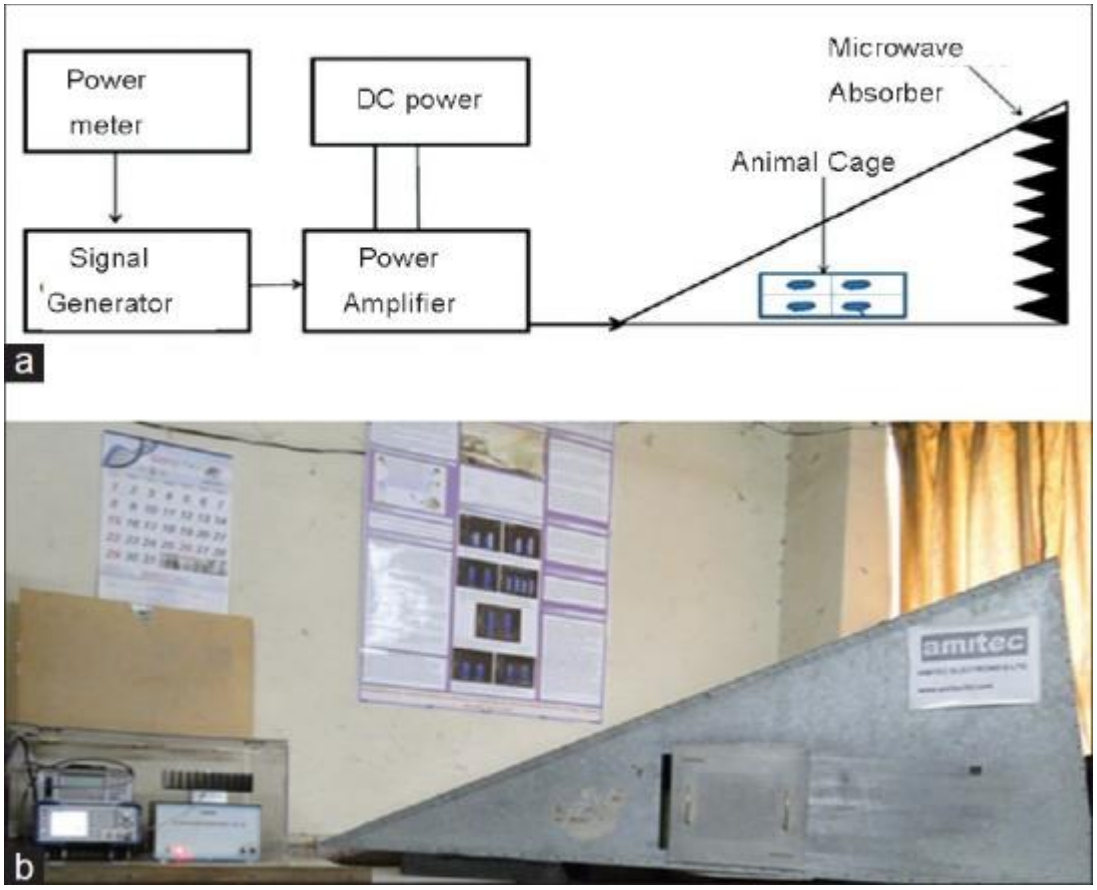
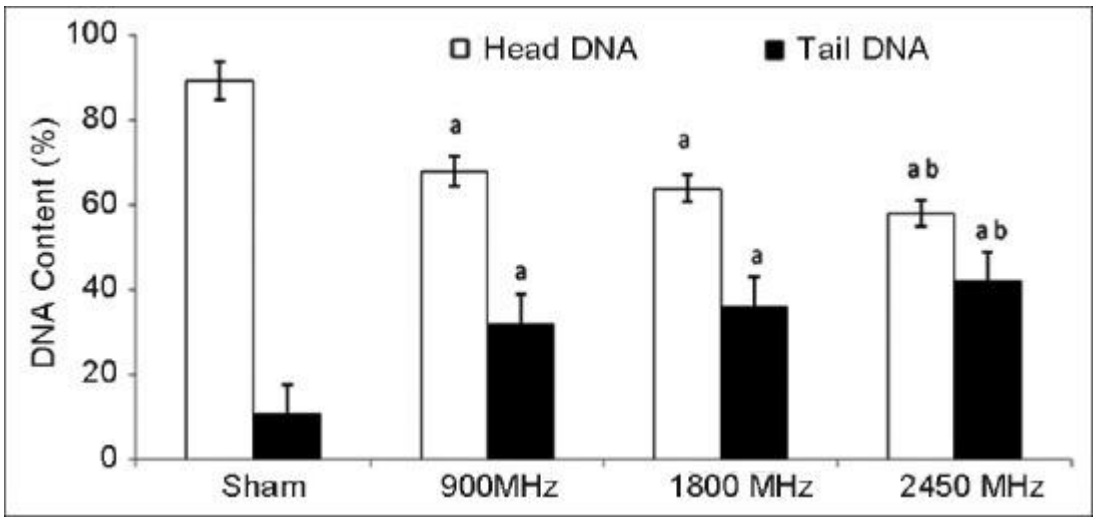
Figures and Tables

Figure 1



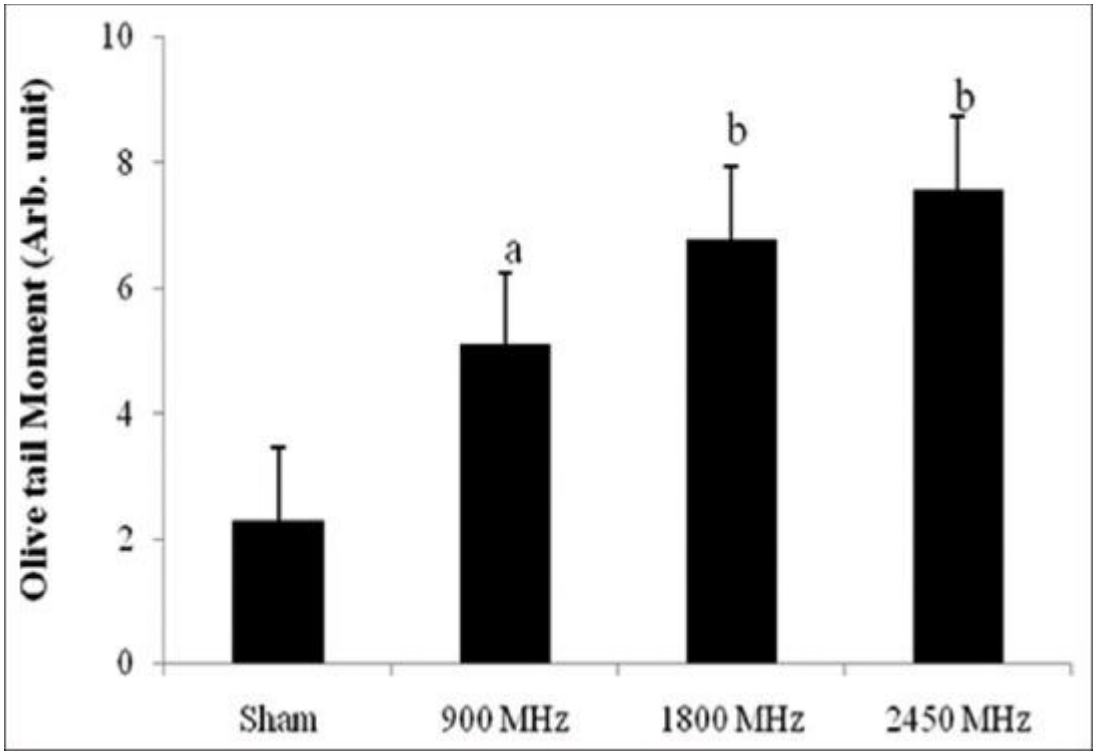
(a) Schematic diagram of microwave exposure setup (b) Photograph of gigahertz transversal electromagnetic cell

Figure 2



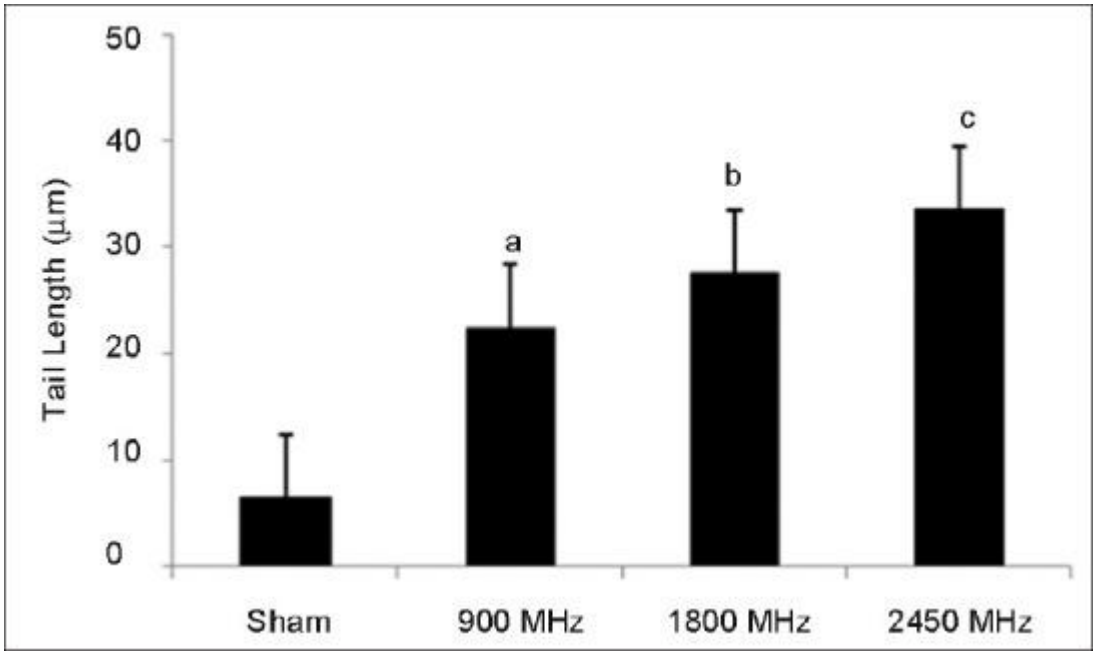
Percent deoxyribonucleic acid in head and tail after 30 days of exposure to microwave radiation. Values are expressed as mean±SE (6 animals per group) Significantly different from [a]sham exposed (*P*<0.001) and [b]900 MHz (*P*<0.001)

Figure 3



Olive tail moment after 30 days of exposure to microwave radiation. Values are expressed as mean±SE (6 animals per group) Significantly different from sham exposed group [a]*P* <0.05 and [b]*P* <0.01

Figure 4



Tail length (in µm) after 30 days of exposure to microwave radiation. Values are expressed as mean±SE (6 animals per group) Significantly different from sham exposed group [a]*P* <0.05, [b]*P* <0.01 and [c]*P* <0.001

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- Abstract

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Drosophila oogenesis as a bio-marker responding to EMF sources.

Margaritis LH[1], Manta AK, Kokkaliaris KD, Schiza D, Alimisis K, Barkas G, Georgiou E, Giannakopoulou O, Kollia I, Kontogianni G, Kourouzidou A, Myari A, Roumelioti F, Skouroliakou A, Sykioti V, Varda G, Xenos K, Ziomas K.

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Abstract

The model biological organisms Drosophila melanogaster and Drosophila virilis have been utilized to assess effects on apoptotic cell death of follicles during oogenesis and reproductive capacity (fecundity) decline. A total of 280 different experiments were performed using newly emerged flies exposed for short time daily for 3-7 d to various EMF sources including: GSM 900/1800 MHz mobile phone, 1880-1900 MHz DECT wireless base, DECT wireless handset, mobile phone-DECT handset combination, 2.44 GHz wireless network (Wi-Fi), 2.44 GHz blue tooth, 92.8 MHz FM generator, 27.15 MHz baby monitor, 900 MHz CW RF generator and microwave oven's 2.44 GHz RF and magnetic field components. Mobile phone was used as a reference exposure system for evaluating factors considered very important in dosimetry extending our published work with D. melanogaster to the insect D. virilis. Distance from the emitting source, the exposure duration and the repeatability were examined. All EMF sources used created statistically significant effects regarding fecundity and cell death-apoptosis induction, even at very low intensity levels (0.3 V/m blue tooth radiation), well below ICNIRP's guidelines, suggesting that Drosophila oogenesis system is suitable to be used as a biomarker for exploring potential EMF bioactivity. Also, there is no linear cumulative effect when increasing the duration of exposure or using one EMF source after the other (i.e. mobile phone and DECT handset) at the specific conditions used. The role of the average versus the peak E-field values as measured by spectrum analyzers on the final effects is discussed.

KEYWORDS:

Apoptosis; DECT base; DECT handset; Drosophila; EMFs; MW oven; Wi-Fi; baby monitor; blue tooth; mobile phones; reproduction

Effect of long-term exposure of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on testes functions.

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Abstract

The aim of this study was to investigate long-term effects of radiofrequency radiation (RFR) emitted from a Wireless Fidelity (Wi-Fi) system on testes. The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (n: 8) and exposure (n: 8). Rats in the exposure group were exposed to 2.4 GHz RFR radiation for 24 h/d during 12 months (1 year). The same procedure was applied to the rats in the sham control group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and reproductive organs were removed. Motility (%), concentration (×10(6)/mL), tail defects (%), head defects (%) and total morphologic defects (%) of sperms and weight of testes (g), left epididymis (g), prostate (g), seminal vesicles (g) were determined. Seminiferous tubules diameter (μm) and tunica albuginea thickness (μm) were also measured. However, the results were evaluated by using Johnsen's score. Head defects increased in the exposure group ($p < 0.05$) while weight of the epididymis and seminal vesicles, seminiferous tubules diameter and tunica albuginea thickness were decreased in the exposure group ($p < 0.01$, $p < 0.001$, $p < 0.0001$). However, other alterations of other parameters were not found significant ($p > 0.05$). In conclusion, we observed that long-term exposure of 2.4 GHz RF emitted from Wi-Fi (2420 μW/kg, 1 g average) affects some of the reproductive parameters of male rats. We suggest Wi-Fi users to avoid long-term exposure of RF emissions from Wi-Fi equipment.

KEYWORDS:

2.4 GHz Wi-Fi; electromagnetic fields; long-term exposure; radiofrequency; reproduction; testes

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The Effect of Electromagnetic Radiation on the Rat Brain: An Experimental Study

Elektromanyetik Dalgaların Sıçan Beyin Dokusu Üzerine Etkileri: Deneysel Çalışma

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ABSTRACT

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum.

MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and 2450 MHz. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months.

RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1β level was significantly increased in the EMR groups in the brain stem.

CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.

KEYWORDS: Electromagnetic waves, Brain tissue, Oxidative stress, Active caspase-3

ÖZ

AMAÇ: Bu çalışmanın amacı, frontal korteks, beyin sapı ve serebellumda elektromanyetik dalgaların yapısal değişikliklerini araştırmaktır.

YÖNTEM ve GEREÇLER: 24 Wistar Albino sıçan rastgele 4 gruba bölündü: grup 1 kontrol grubu ve grup 2-4 900, 1800 ve 2450 MHz elektromanyetik radyasyona maruz kaldı. Sıçanların kafaları 900, 1800 ve 2450 MHz mikrodalgalara 2 ay boyunca her gün 1 saat maruz bırakıldı.

BULGULAR: Kontrol grubunda histopatolojik değişiklikler, beyin sapında ve frontal kortekste normal bulunurken elektromanyetik radyasyon uygulanan gruplarda ciddi dejeneratif değişiklikler, küçülmüş sitoplazma ve geniş koyu piknotik nükleus mevcuttu. Biyokimyasal analizde total antioksidatif kapasite seviyesi elektromanyetik grupta anlamlı olarak azalmış bulunurken, total oksidatif kapasite ve oksidatif stres indeksi seviyeleri frontal korteks, beyin sapı ve serebellumda anlamlı olarak artmış bulundu. Elektromanyetik grupta beyin sapında IL-1β seviyesi anlamlı olarak artmıştı.

SONUÇ: Elektromanyetik radyasyon beyin sapında, serebellumda ve frontal kortekste yapısal değişikliklere neden olmakta ve oksidatif stres ve inflamatuvar sitokin sistemini bozmaktadır. Bu bozulma kanser oluşumuna ve bu bölgelerin fonksiyon kaybını içeren hastalıklara neden olabilir.

ANAHTAR SÖZCÜKLER: Elektromanyetik dalga, Beyin dokusu, Oksidatif stres, Aktif kaspaz-3

INTRODUCTION

The frequent using of mobile and cordless phones in our daily life has led to concern regarding possible health effects, cancer risk in particular, from frequent exposure to radiofrequency radiation. However, scientific evidence on a possible mobile phone–cancer relation has still not clear enough. Therefore, investigators have still been showing high performance to clarify this topic (3). Electromagnetic radiation (EMR) is emitted by both cellular mobile phones and their base stations. Unfortunately, exposure to EMR may have

detrimental effects on the body, depending on the frequency and power of the EMR (27). Analogue phones use frequencies of 400–450 MHz, and digital mobile phones use frequencies of 850–900 MHz (similarly used in our study), and 1850–1990 MHz, whereas microwave ovens use a frequency of 2450 MHz (24).

During embryogenesis, and throughout the lifespan of a multi-cellular organism, apoptosis plays a key role in normal tissue homeostasis (1,8). Perturbations in apoptotic pathways can cause human disease (30). There are several markers currently used to detect apoptosis. In particular, the active (cleaved) caspase-3 is a well-characterized component typically observed during apoptosis, making it an ideal marker for this cellular process (9). Defects in apoptosis signaling pathways are common in cancer cells. Since apoptosis eliminates cells with damaged DNA or dysregulated cell cycle (as is the case for cells with increased malignant potential), deficits in apoptotic signaling may play an important role in tumor initiation (3). Deficits in apoptosis may also promote tumor progression and metastasis by enhancing the survival of tumor cells during transit through the bloodstream and growth in ectopic tissues (17).

Dasdag et al. (4) intended to examine the effects of 900 MHz mobile phone exposure on p53 in the rat brain. They found that 900 MHz microwave radiation emitted from mobile phones do not affect these apoptotic parameters (4). Leszczynski et al. (18) reported that mobile phone exposure can alter heat shock protein-27 (hsp27) by causing a transient increase in its phosphorylation. Hsp27 has many known functions that suggest that mobile phone radiation-induced activation of hsp27 may (i) promote brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) enhance the permeability of the blood-brain barrier by stabilizing stress fibers of endothelial cells.

EMR or radiofrequency fields of cellular mobile phones may also affect individuals by increasing free radicals, which enhance lipid peroxidation, or by promoting oxidative stress by changing the antioxidant defense systems of human tissues (24). Oxidative stress is caused by relatively high levels of toxic reactive species, consisting mostly of reactive oxygen species (ROS), reactive nitrogen species (RNS), and the antioxidative defense mechanisms (23). Electromagnetic radiation causes oxidative stress, which in turn induces apoptosis. The signal for apoptosis is possibly generated via lipid peroxidation when radiation acts on cell membranes (23).

IL-1β, an important component of the cytokine network, and is one of the inflammatory cytokines that introduces prostaglandin E1, matrix metalloproteinase-3, nitrous oxide (NO), and other substances into the intervertebral discs (22). IL-1β is a pro-inflammatory cytokine elicited following traumatic brain injury; behavioral outcome is improved when this cytokine is decreased (27).

We investigated the effects of cell phone exposure on the antioxidants, oxidants, oxidative stress index, interleukin-1β levels and histological structure of rat brain tissue, caspase-3 immunoreactivity in this study.

MATERIAL and METHODS

Animal Model

Twenty-four male Spraque-Dawley rats (8-week-old, 150–200 g body weight) obtained from the Laboratory Animal Production Unit of Suleyman Demirel University were used in the study. The animals were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Suleyman Demirel University, Animal Ethical Committee. They were kept in a temperature- and humidity-controlled environment (temperature, 24–26°C; humidity, 55–60%), on a 12:12-h light/darkness cycle for 1 week before the start of experiments. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Experimental Design

The animals were randomly divided into four equal groups (consisting of 6 rats each): Group I rats were used as the control group (without exposure to EMR); these rats were held in the EMR tube for 60 minutes a day for 60 days (two months) under the same environmental conditions. EMR groups: group II rats were exposed to 900 MHz, group III rats were exposed to 1800 MHz, and group IV rats were exposed to 2450 MHz. All of the rats in the EMR groups were exposed to EMR from the generator for 60 minutes a day for 60 days. The EMR exposure time was from 11:00–12:00 a.m. on each day. At the end of the study, the rats in all of the groups were anesthetized with intraperitoneal administration of ketamine (50 mg/kg)-xylazine (6 mg/kg) after the last dose exposure to EMR. The rats were sacrificed by collecting blood samples from the posterior vena cava. After removing the cranial bones, cerebral cortex and brain stem removed and the brain stem, right frontal cortex and cerebellum tissue were kept in -86°C for biochemical study. Left frontal cortex, cerebellum and brain stem were taken in neutral buffered formalin solution for histopathological study. Histopathological evaluation of left cerebellum was unsuccessful due to staining failure.

Exposure Device

Radiation for the study (900, 1800, 2450 MHz) was provided by an electromagnetic generator continuously for 60 min each day for 2 months (peak power, 2 W; average power density, 1.04 mW/cm^2). The predicted average specific absorption rate (SAR) value was measured at 1.04 W/kg. The power density measurements were made with an electromagnetic field meter (Holaday Industries Inc.) produced at the electromagnetic compatibility (EMC) laboratory of the School of Electronic Engineering (Suleyman Demirel University, Isparta, Turkey). The exposure system consisted of a plastic tube cage (length: 12 cm, diameter: 5.5 cm) and a dipole antenna. The entire body of each rat was positioned in close contact above the dipole antenna, and the tube was ventilated from head-to-tail to decrease the stress of the rat while in the tube.

Biochemical Analysis Tissue Sampling and Homogenization

On the last day of the study, brains were removed immediately after euthanization to determine total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI). Prior to biochemical assays, all tissues were weighed and placed in empty glass tubes. Ten milliliters of 140 mM KCl solution per 1 g of tissue were added to each tube containing tissue samples, and the tissue was homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2800 *g* for 10 min at 4°C, placed in labeled vials and stored at −80°C. Microprotein levels were measured using the Lowry method (20).

Measurement of TAC

TAC levels of all tissues were measured using a novel automated colorimetric measurement method developed by Erel (6,7). This method is based on the Fenton reaction, which involves the colorless substrate *O*-dianisidine reacting with the hydroxyl radical to produce the dianisyl radical, which is bright yellowish-brown. Upon the addition of a plasma sample, hydroxyl radicals present in the reaction mix initiate oxidative reactions that are suppressed by the antioxidant components within the homogenates. This prevents the color change, providing an effective measure of the total antioxidant capacity of the plasma. The assay has excellent precision values (<3% error). Assay results are expressed as µmol Trolox equiv/g protein.

Measurement of TOS

TOS levels of all tissues were determined using a novel automated measurement method developed by Erel (7). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. Glycerol molecules present in the reaction medium enhance the oxidation reaction. In an acidic medium with xylenol orange, the ferric ion forms a colored complex. Spectrophotometric measurement of the color intensity represents the total amount of oxidant molecules present in the sample. Hydrogen peroxide (H_2O_2) is used to calibrate the assay, and the results are expressed as micromolar H_2O_2 equivalents per g protein (µmol H_2O_2 equiv/g protein).

OSI Calculation

OSI is the percent ratio of TOS to TAC and an indicator of the degree of oxidative stress (7). The OSI value was calculated according to the formula: OSI = ((TOS, µmol H_2O_2 Equiv/g protein)/(TAC, µmol Trolox equiv/g protein)).

Interleukin-1β Determination

Brain stem tissue weighed in phosphate buffer (pH 7.4) was diluted in 10 volumes. Tissue samples were separated by centrifugation (Eppendorf 5415-R (Germany) brand refrigerated centrifuge) and 4000 rev/min for 15 min, and the supernatant from the IL-1β was studied. The supernatants were stored at −20°C until the cytokine assays were performed. Cytokines in the supernatant of tissue homogenates were measured using commercially available enzyme-linked immunosorbent assay according to the manufacturer's instructions. The assay kits for IL-1β were purchased from RayBio mark (USA).

Histopathologic Evaluation

Frontal cortex and brainstem tissues were harvested and fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at a thickness of 5 µm, and then stained with hematoxylin and eosin (H&E). Sections were photographed and were evaluated using a bright-field microscope (Optiphot 2; Nikon, Tokyo, Japan).

Microscopic Examination

Tissue sections were examined using a bright-field microscope, and the number of neurons was counted within random high-power fields using a Nikon Optiphot-2 light microscope fitted with a square graticule in the eyepiece (eyepiece x10, objective x40, a total side length of 0.25 μm^2). The number of neurons was assessed by counting the number of cells in the frontal cortex and brainstem in 400 high-power fields. The number of neurons/μm^2 in each region was determined. The tissue compartments were used to record neuron distribution in the frontal cortex and brain stem tissues.

Immunohistochemistry

Frontal cortices and brain stems were fixed in 10% neutral buffered formalin solution, embedded in paraffin, and sectioned at a thickness of 5 µm. Immunocytochemistry was performed according to the ABC technique described previously (13). The procedure involved the following steps: (1) incubation in 3% H_2O_2 in distilled water for 30 min to inhibit endogenous peroxidase activity; (2) washing in distilled water for 10 min; (3) incubation in normal goat serum (DAKO X 0907, Carpinteria, CA) with phosphate-buffered saline (PBS) (diluted 1:4) to block non-specific binding of antibodies; (4) incubation in rabbit polyclonal anti-Caspase-3 antibody (1:50; ab13847, Abcam, USA) for 1 h at room temperature; (5) washing in PBS 3 × 3 min; (6) incubation in biotinylated anti-mouse IgG (DAKO LSAB 2 Kit); (7) washing in PBS 3 × 3 min; (8) incubation in ABC complex (DAKO LSAB 2 Kit); (9) washing in PBS 3 × 3 min; (10) detection of peroxidase using an aminoethylcarbazole substrate kit (AEC kit; Zymed Laboratories); (11) washing in tap water for 10 min, followed dehydration; (12) hematoxylin staining to visualize the nuclei; and (13) mounted in DAKO Paramount. All dilutions and washes between steps were performed using PBS and were carried out at room temperature unless otherwise specified. As a negative control, the primary antibody was replaced with PBS.

Immunohistochemical staining was scored in a semiquantitative manner to determine differences between the control group and the experimental groups. Weak (±), mild (+), moderate (++), strong (+++), and very strong (++++) signals was observed and recorded. This analysis was performed in

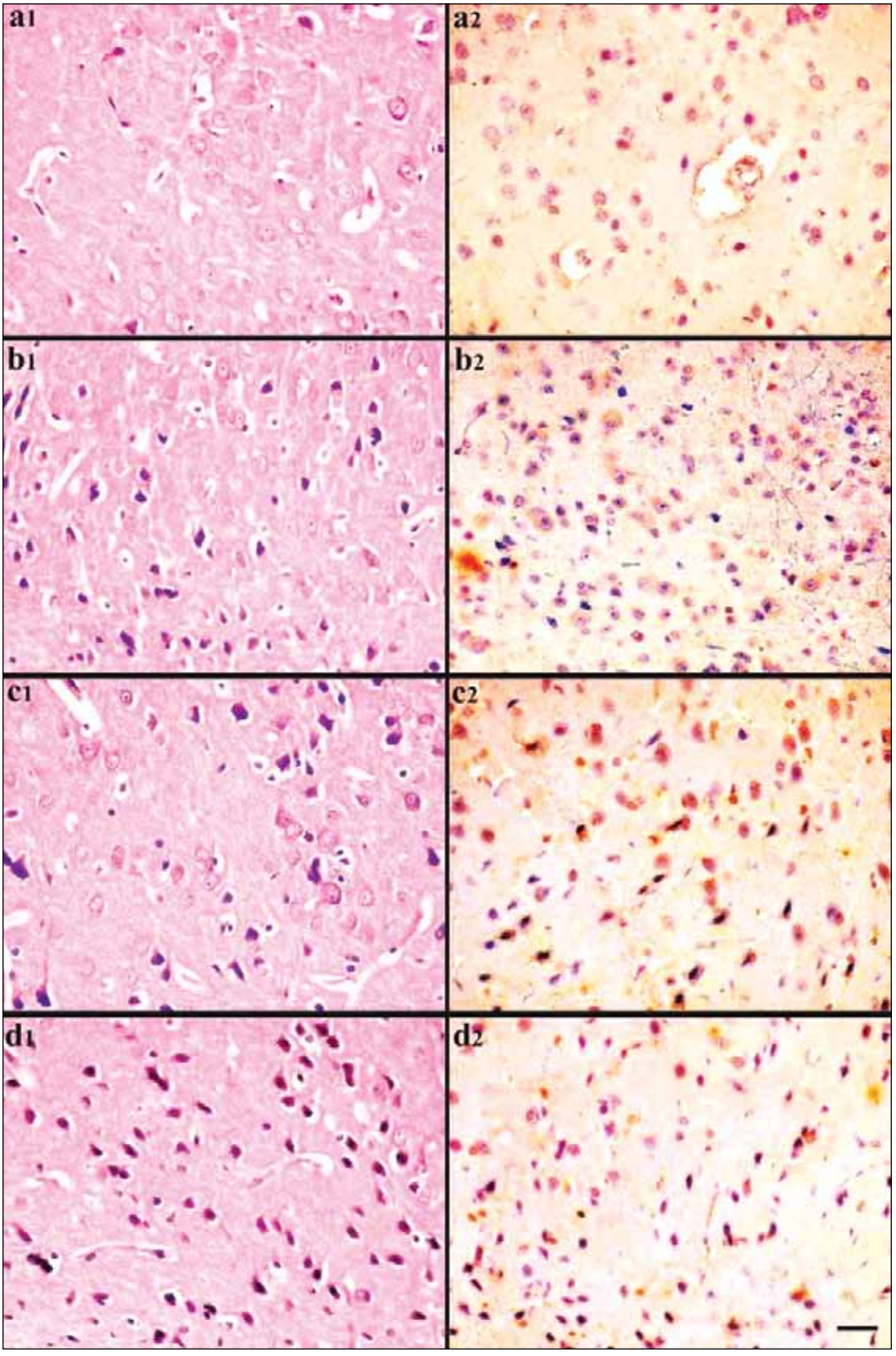

Figure 1: Representative light microphotographs showing the morphology (a1–d1) and apoptosis (a2–d2) of the frontal cortex tissue in control and EMR groups (900, 1800, 2450 MHz) by hematoxylin-eosin and caspase-3 immunohistochemistry. (Immunoperoxidase, haematoxylin counterstain, scale bar, 50 µm) .

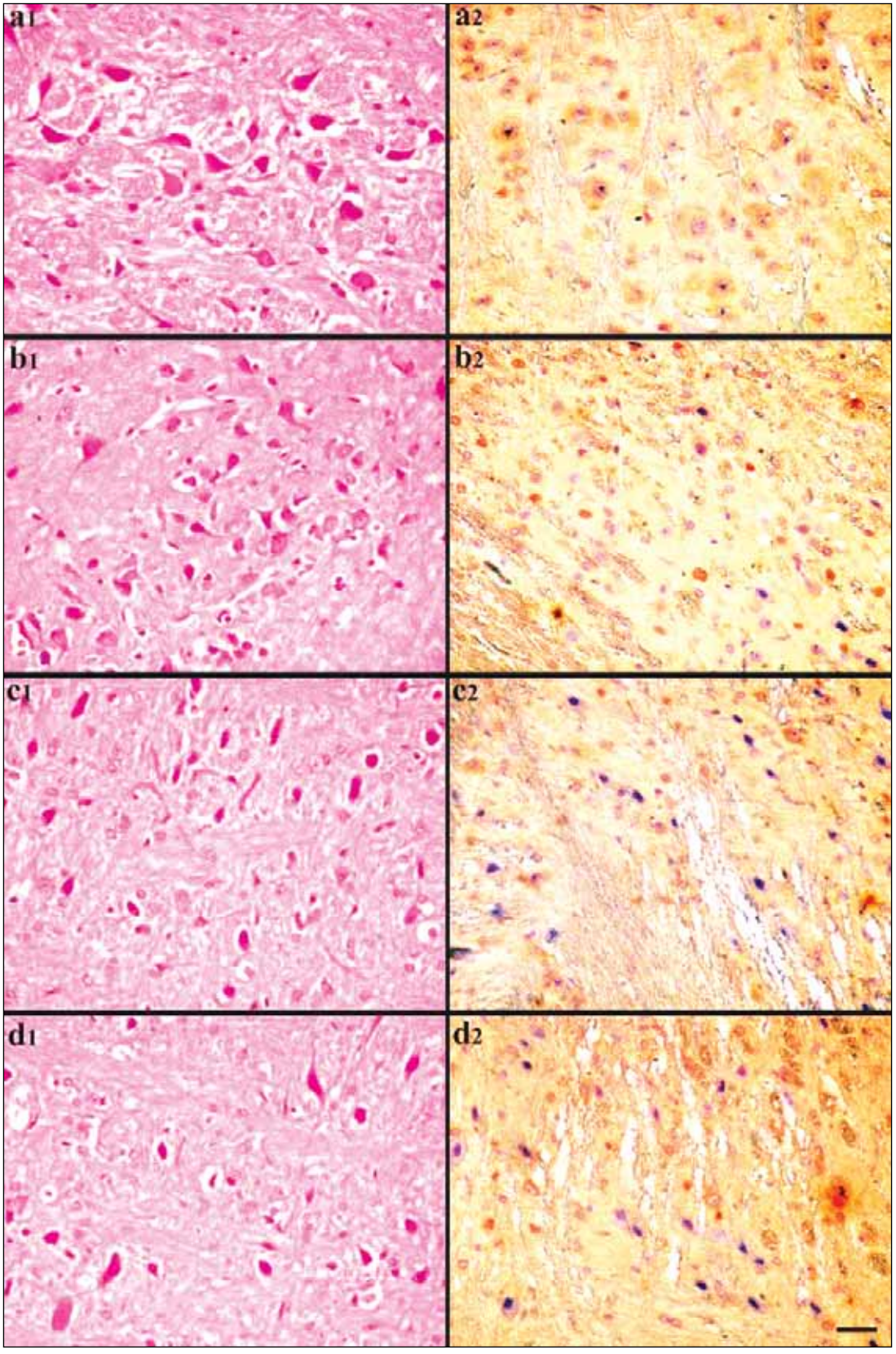

Figure 2: Representative light microphotographs showing the morphology (a1–d1) and apoptosis (a2–d2) of the brain stem tissue in control and EMR groups (900, 1800, 2450 MHz) by hematoxylin-eosin and caspase-3 immunohistochemistry. (Immunoperoxidase, haematoxylin counterstain, scale bar, 50 µm) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16.

at least 8 areas from the frontal cortex and brain stem, in 2 sections from each animal at x400 magnification.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Data from controls and each of the groups were compared using the Mann–Whitney non-parametric test for independent samples. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc. Chicago, IL, USA) and a $p < 0.05$ was considered significant.

RESULTS

Histopathological Findings

The morphology of neurons in the frontal cortex and brain stem were normal in H&E-stained sections taken from the control group (Figure 1A1; 2A1). In the EMR groups (900, 1800, and 2450 MHz), the most consistent observations were severe degenerative changes, shrunken cytoplasm, and extensively dark pyknotic nuclei in neurons of the frontal cortex and brain stem tissues (Figure 1B1, C1, D1; 2B1, C1, D1). In the EMR groups (900 and 1800 MHz) frontal cortex and brain stem tissues, the intensity of neuronal changes was less than in the EMR group (2450 MHz). The number of neurons in the frontal cortex and brain stem tissues of the EMR groups (900, 1800, 2450 MHz) were also significantly less than in the control group ($P < 0.05$, $P < 0.01$ and $P < 0.001$) (Table I).

Immunohistochemical Findings

Weak caspase-3 immunoreactivity was observed in the cytoplasm of neurons in control rats (Figure 1A2; 2A2). Light micrographs of caspase-3 immunohistochemistry revealed apoptotic neurons after EMR exposure. The caspase-3 signal was more intense in degenerating neurons of the frontal cortex and brain stem tissues following EMR exposure (Figure 1B2–D2; 2B2–D2). In the cytoplasm of neurons in EMR (2450 MHz) group, the strong caspase-3 immunoreactivity was observed (Figure 1D2; 2D2) (Table II).

Biochemical Analysis

Determination of total antioxidative capacity (TAC), total oxidative status (TOS) and Oxidative Stress Index (OSI):

The determination of TAC, TOS and OSI values in the frontal cortex are shown in Table III. The frontal cortex in the EMR groups TAC were lower than in the control group ($p<0.05$), TOS and OSI were higher than in the control group ($p<0.05$). In the EMR groups, the group II frontal cortex TAC was significantly different from that of groups III and IV ($P<0.05$), whereas the group III TAC was not significantly different from that of group IV ($P>0.05$). The group IV frontal cortex TOS was significantly different from that of groups II and III ($P<0.05$), whereas the group II TOS was not significantly different from that of group III ($P>0.05$). For the OSI comparisons, the OSI in frontal cortex group II was significantly different from that of group III and IV ($P<0.05$), and the group III OSI was not significantly different from that in group IV ($P>0.05$).

The determination of TAC, TOS and OSI values in the brain stem are shown in Table IV. The brainstem in the EMR groups TAC were lower than in the control group ($p<0.05$), TOS and OSI were higher than in the control group ($p<0.05$). In the EMR groups, the group II TAC was not significantly different from that of groups III and IV ($P>0.05$). The group II TOS was significantly different from that of groups II and III ($P<0.05$), whereas the group III TOS was not significantly different from that of group IV ($P>0.05$). For the OSI comparisons, the OSI group II was not significantly different from that of group III and IV ($P>0.05$).

The determination of TAC, TOS and OSI values in the cerebellum are shown in Table V. The cerebellum in the EMR groups TAC were lower than in the control group ($p<0.05$), TOS and OSI were higher than in the control group ($p<0.05$). In the EMR groups, the group II TAC was not significantly different from that of groups III ($P>0.05$), whereas the group IV TAC was significantly different from that of group II ($P<0.05$). The group II TOS was significantly different from that of groups III ($P<0.05$), whereas the group II TOS was not significantly different from that of group IV ($P>0.05$). For the OSI comparisons, the OSI group II was not significantly different from that of group III and IV ($P>0.05$), and the group III OSI was not significantly different from that in group IV ($P>0.05$).

Measurement of Brain Stem IL-1β levels

The brain stem IL-1β levels are showed in table 4. IL-1β levels in the EMR groups were significantly different from those in

Table I: The Numbers (Number/mm^2) of Neurons in the Frontal Cortex and Brain Stem Tissue of Control and EMR Groups (900, 1800, 2450 MHz)

Groups	Frontal cortex	Brain stem
Control	77.38±5.13	35.31±1.54
900 MHz	63.82±4.64[a]	28.12±1.32[a]
1800 MHz	51.44±3.86[b]	23.14±1.11[b]
2450 MHz	42.16±2.98[c]	18.12±0.94[c]

Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, n = 6 for each group; [a]P<0.05 compared to A group, [b]P<0.01 compared to A group, [c]P<0.001 compared to A group.

Table II: Semiquantitative Comparison of the Intensity in Neurons of Caspase-3 Immunoreactivity in the Frontal Cortex and Brain Stem Tissue of Control and EMR Groups (900, 1800, 2450 MHz), (n: 6 for Each Group)

	Frontal cortex	Brain stem
Control	±	±
900 MHz	+	+
1800 MHz	++	++
2450 MHz	+++	+++

The intensity of the staining was recorded as weak (±), mild (+), moderate (++), strong (+++) and very strong (++++).

the control group (P<0.05). In the EMR groups, group II IL-1β levels were significantly different from those in group III and IV (P<0.05), whereas group III IL-1β levels were not significantly different from those in group IV (P>0.05).

DISCUSSION

The frequent using of mobile phones and base stations in our daily life has led to concern regarding possible health effects, cancer risk in particular, from frequent exposure to radiofrequency radiation. However, scientific evidence on a possible mobile phone–cancer relation has still not clear enough (3). There fore we aimed to investigate experimentally the biological effect of EMR on the cranial structure. In this study, we demonstrated that EMR affect the oxidative stress, levels of cytokine and induction of the apoptosis on the brain tissue.

Cancer cells often exhibit deficits in apoptotic signaling pathways. These deficits may play a significant role in tumor initiation because cells with damaged DNA or dysregulated cell cycle (i.e., cells with increased malignant potential) are normally eliminated (17). Moreover, impaired apoptotic function may enhance tumor progression and promote metastasis by enhancing the ability of tumor cells to survive in the circulatory system and to grow in ectopic tissue sites (17). Therefore, the result of this study is of importance in terms of its implication on the dysregulation of apoptotic machinery. Because we found that 900/1800/2400 MHz radiation used in this study altered the number of apoptotic cells, this may potentially be dangerous in the networking of the brain cells.

Radiation is known to induce oxidative stress, which in turn activates the apoptotic pathway (23). Oxidative stress is a cellular or physiological condition of elevated concentrations of reactive oxygen species that cause molecular damage to vital structures and functions (32). It has been reported that the effects of radiation on cell membranes induces apoptotic signal via lipid peroxidation (23). Radiation may also mediate

Table III: The Levels of Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Frontal Cortex Groups

Parameters	Control	900MHz	1800MHz	2450MHz
TAC	0.90±0.05	0.59±0.01	0.74±0.02	0.78±0.03
TOS	3.29±0.35	4.77±0.40	4.71±0.24	6.15±0.99
OSI	418.48± 41.38	808.52± 69.15	629.38± 28.78	678.01± 83.53

The value represent the mean±SD.
TAC compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800/2450 MHz (p<0.05), 1800- 2450 MHz (p>0.05).*
TOS compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800 MHz (p>0.05), 900- 2450 MHz (p<0.05), 1800-2450 MHz (p<0.05).*
OSI compares; *Control- 900/1800/2450 MHz (p<0.05), 900-1800/ 2450 MHz (p<0.05), 1800-2450 MHz (p>0.05) Arbitrary Units (AU).*

Table IV: The Levels of Interleukin-1B (Il-1B), Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Brain Stem Groups

Parameters	Control	900MHz	1800MHz	2450MHz
IL-1β	5061.57± 418.75	8189.23± 1151.21	6055.58± 648.34	6039.02± 710.64
TAC	0.37±0.01	0.26±0.02	0.27±0.01	0.28±0.00
TOS	2.22±0.13	3.71±0.30	3.03±0.14	2.68±0.11
OSI	661.82± 36.16	1094.94± 108.29	1080.30± 78.29	1074.16± 63.23

The value represent the mean±SD.
IL-1β compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900-1800/2450 MHz (p<0.05), 1800-2450 MHz (p>0.05).*
TAC compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800/2450 MHz (p>0.05), 1800- 2450 MHz (p>0.05).*
TOS compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800/2450 MHz (p<0.05), 1800-2450 MHz (p<0.05).*
OSI compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900-1800/ 2450 MHz (p>0.05), 1800-2450 MHz (p>0.05) Arbitrary Units (AU).*

Table V: The Levels of Total Antioxidatif Capacity (TAC), Total Oxidant Status (TOS) and Oxidant Stres Index (OSI) in Cerebellum Groups

Parameters	Control	900MHz	1800MHz	2450MHz
TAC	0.58±0.08	0.27±0.01	0.27±0.02	0.24±0.01
TOS	2.30±0.21	3.01±0.35	2.59±0.18	2.59±0.20
OSI	400.85± 80.75	1110.03± 146.43	950.38± 94.75	1077.33± 154.62

The value represent the mean±SD.
TAC compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800 MHz (p>0.05), 900-2450 MHz (p<0.05), 1800-2450 MHz (p>0.05).*
TOS compares; *Control- 900/ 1800/ 2450 MHz (p<0.05), 900- 1800 MHz (p<0.05), 900- 2450 MHz (p>0.05), 1800-2450 MHz (p>0.05).*
OSI compares; *Control- 900/1800/2450 MHz (p<0.05), 900-1800/ 2450 MHz (p>0.05), 1800-2450 MHz (p>0.05) Arbitrary Units (AU).*

its apoptotic effects by increasing oxidative stress (24). An increase in free radical formation caused by radiations from mobile phones has been reported in other tissues (14–16). Ilhan et al. demonstrated that mobile phones biochemically cause oxidative damage by increasing the levels of NO, MDA, XO, and ADA activities in rat brain used as a model for EMR exposure. These continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT) (24). Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue (24).

The high number of antioxidant components in tissues hinders individual measurement of each antioxidant component (5). In addition, because of the interaction between various antioxidants, examining one of them separately from the others may not accurately reflect their combined effect (5). Therefore, TAC and TOS, developed by Erel (6,7), are 2 biochemical parameters that seem to appropriately represent the overall oxidant and antioxidant status resulting from antioxidant intake, or production and consumption by increasing oxidative stress level (5).

Hardel et al. (10,11) and Lonn et al. (19) reported that exposure to mobile phone radiation increased the incidence of brain tumors and acoustic neurinoma, depending on the duration of mobile phone use. Belyaev et al. (2) investigated whether rat brain exposure to microwaves of the global system for mobile communication (GSM) induces 1) DNA breaks and 2) changes in chromatin conformation and gene expression. Leszczynski et al. (18) reported that mobile phone exposure results in a transient increase in phosphorylation of hsp27. The function of hsp27 suggests that mobile phone radiation-induced activation of this protein may (i) increase the likelihood of brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) increase blood-brain-barrier permeability by stabilizing endothelial cell stress fibers (18). Finally, Dasdag et al. (3) showed that the apoptotic process can be affected by radiofrequency radiation. In this study we demonstrated that in the cytoplasm of neurons in EMR groups, the caspase-3 immunoreactivity which is a well-known feature of typical apoptosis was observed strongly especially in the 2450 MHz group.

Inflammatory cytokines, including TNF-α, IL-1β, and IL-6, are released by microglial cells during neuropathological states and in response to various neuropathological stimuli (21,25). IL-1β is a pro-inflammatory cytokine and an important upstream regulator of the inflammatory response that occurs following trauma (27). IL-1β levels in the cerebrospinal fluid (CSF) positively correlates with high intracranial pressure and poor outcomes in human patients (29). IL-1β possibly does not directly cause neuronal injury, but may instead act through astrocytes (26) by responding to harmful stimuli (31).

We investigated the effect of EMR on IL-1β because, as a mediator of the pro-inflammatory response and predictor of clinical outcome, it plays a key role in injury response. Hirose et al. (12) demonstrated that there was no differences of IL-1β level between control and 1950 MHz groups in rat brain. Also Rasouli et al (27) demonstrated no differences in IL-1β level between pulsed electromagnetic fields treated and control groups in brain homogenates after traumatic brain injury in rats. In this study, IL-1β level was significantly increased in brain stem in EMR groups compared to the control group.

CONCLUSION

Biochemical analysis of this study demonstrated that TAC level was significantly decreased in EMR groups compared to control group while TOS and OSI level were significantly increased. This result revealed that EMR is affective on the oxidant-antioxidant system. The frontal cortex was significantly more affected in 900 MHz EMR. There was not significant difference between other groups. Also in the brain stem and frontal cortex there was not significantly differences between EMR groups. IL-1β level was significantly increased at brain stem in EMR group compared to control group. EMR in terms of IL-1β levels most affected group was 900 MHz. There were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei and less neuron in the EMR groups compared to control groups. The most affected EMR group was the 2450 MHz group. In the frontal cortex and brain stem of the EMR groups, caspase-3 immunoreactivity was increased compared to the control group. The most affected group was again the 2450 MHz group.

DECLARATION of INTEREST

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Effects of acute exposure to WIFI signals (2.45GHz) on heart variability and blood pressure in Albinos rabbit.

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Abstract

Electrocardiogram and arterial pressure measurements were studied under acute exposures to WIFI (2.45GHz) during one hour in adult male rabbits. Antennas of WIFI were placed at 25cm at the right side near the heart. Acute exposure of rabbits to WIFI increased heart frequency (+22%) and arterial blood pressure (+14%). Moreover, analysis of ECG revealed that WIFI induced a combined increase of PR and QT intervals. By contrast, the same exposure failed to alter maximum amplitude and P waves. After intravenously injection of dopamine (0.50ml/kg) and epinephrine (0.50ml/kg) under acute exposure to RF we found that, WIFI alter catecholamines (dopamine, epinephrine) action on heart variability and blood pressure compared to control. These results suggest for the first time, as far as we know, that exposure to WIFI affect heart rhythm, blood pressure, and catecholamines efficacy on cardiovascular system; indicating that radiofrequency can act directly and/or indirectly on cardiovascular system.

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KEYWORDS:

Arterial pressure (PA); Dopamine; Efficacy of catecholamines; Electrocardiogram (ECG); Epinephrine; Heart rate (HR)

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Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats

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Abstract

Introduction:

Melatonin has been considered a potent antioxidant that detoxifies a variety of reactive oxygen species in many pathophysiological states of eye. The present study was designed to determine the effects of Wi-Fi exposure on the lens oxidant, antioxidant redox systems, as well as the possible protective effects of melatonin on the lens injury induced by electromagnetic radiation (EMR).

Materials and Methods:

Thirty-two rats were used in the current study and they were randomly divided into four equal groups as follows: First and second groups were cage-control and sham-control rats. Rats in third group were exposed to Wi-Fi (2.45 GHz) for duration of 60 min/day for 30 days. As in the third group, the fourth group was treated with melatonin. The one-hour exposure to irradiation in second, third and fourth took place at noon each day.

Results:

Lipid peroxidation levels in the lens were slightly higher in third (Wi-Fi) group than in cage and sham control groups although their concentrations were significantly ($P < 0.05$) decreased by melatonin supplementation. Glutathione peroxidase (GSH-Px) activity was significantly ($P < 0.05$) lower in Wi-Fi group than in cage and sham control groups although GSH-Px ($P < 0.01$) and reduced glutathione ($P < 0.05$) values were significantly higher in Wi-Fi + melatonin group than in Wi-Fi group.

Conclusions:

There are poor oxidative toxic effects of one hour of Wi-Fi exposure on the lens in the animals. However, melatonin supplementation in the lens seems to have protective effects on the oxidant system by modulation of GSH-Px activity.

Keywords: Antioxidants, electromagnetic ration, glutathione, lens, oxidative stress

People are intensely exposed to electromagnetic radiation particularly for the last two decades. Electromagnetic radiation (EMR) signals consist of extremely low frequency fields (between 1Hz up to 100 kHz) and high frequency fields, in the band of the radio frequency fields (RF, 100 kHz-3GHz) and of the microwaves (MW, above 3 GHz). Besides their use for technological applications (e.g, power lines, mobile phones), the EMFs are today widely used in medicine for diagnostic and therapeutic purposes.[1,2] The World Health Organization Report (2007) and WHO Environmental Health Criteria Report (2007) issued precautions against EMR, considering the increased social and public interest in this subject, based on the epidemiological data, which associates the extra risk of amyotrophic lateral sclerosis, childhood leukemia, adult brain cancer, and miscarriage with EMR exposure of the power line radiation.[3]

The interaction of the biological system and EMR in the molecular level can result in change in cell cycle, induction of cell death, modification of protein expression and mainly oxidative stress.[4,5,6,7] EMR exposure of eye results in overproduction of reactive oxygen species (ROS), which can damage cellular components such as lipids, nucleic acids and proteins.[8] Lipid peroxidation in eye occurs because of elevated levels of ROS with the liberation of reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal.[9] ROS in eye can cause cellular damage by depleting enzymatic and/or non-enzymatic antioxidants triggering progressive dysfunction and eventually genotoxic events.[10,11] ROS are also increasingly generated in the tissues in many vision impairing and blinding diseases including cataracts, glaucoma and retinopathies. Oxidative stress induced by ROS is a major risk factor for senile cataract, particularly nuclear cataract.[9] The level of ROS in eye is usually regulated by intracellular antioxidant defense system. The antioxidant defense system is composed of antioxidant molecules such as reduced glutathione (GSH) and of a variety of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (GSH-Px).[12] The eye lens has also evolved a wide range of protective and repair systems to protect its components from oxidative stress, including high levels of reduced GSH and abundant antioxidant enzymes such as superoxide dismutase and catalase.[13]

Melatonin (N-acetyl-5-methoxytryptamine), also an indoleamine, is a neurohormone secreted primarily by the pineal gland and has an effect on most biological and physiological processes. The biosynthesis of melatonin from serotonin proceeds via two key enzymes, arylalkylamine N-acetyltransferase and hydroxyindole-O-methyltransferase (5-hydroxytrypamine).[14] Melatonin in the eye is synthesized in the crystalline lens cortical fiber cells, retinal photoreceptor cells and ciliary body. Additionally, specific melatonin receptors are found in the retina, coroid, ciliary body, lens, sclera, which are targets for melatonin.[15]

We have recently observed modulator role of melatonin on Wi-Fi-induced oxidative stress in brain and dorsal root ganglion of rats.[16] To our knowledge, there is no report whether Wi-Fi induce oxidative stress in lens of experimental animals and humans. The aim of the present study was to determine the effects of Wi-Fi frequency (2.45 GHz) exposure on the lens oxidant, antioxidant redox systems, as well as the possible protective effects of melatonin on the lens injury induced by the EMR.

Go to:

Materials and Methods

Animals

In this study, 16 weeks old, 32 male albino rats of Wistar strain, weighing 220 ± 20 g were used. The study was approved by Ethical Committee of Suleyman Demirel University. Laboratory animals prepared by Ethical Committee of (Protocol Number; 2011-01). The rats were quarantined before irradiation, housed in individual stainless-steel cages in a pathogen-free environment in a windowless laboratory room with automatic temperature (22 ± 3°C) and lighting controls (12-h light/12-h dark) and fed standard laboratory chow and water ad libitum. Average light intensity was determined to be 4000 lux and humidity was 40 ± 10% in the laboratory. The authors confirm adherence to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research.

Experimental groups

After one week adaptation process, the animals were randomized into four groups of eight rats each. The rats in Group A1 (cage control) were not exposed to electromagnetic radiation (EMR) or did not receive melatonin. The rats in Group A2 (sham control) received an intraperitoneal injection of isotonic saline solution in the same volume and by the same scheme as that used in the group receiving melatonin without EMR exposure. Group B rats (the EMR group) were exposed to 1 hour of EMR from 9 am to 10 am for 30 days. Group C rats (treatment groups) were exposed to EMR in the same manner as Group B and received melatonin treatment. Melatonin was initially dissolved in 0.1 ml of dimethyl sulfoxide (DMSO) and then diluted with physiological saline solution. Melatonin was administered intraperitoneally at a dose of 10 μg/kg and in a 0.1 ml volume of DMSO. The injection was administered 24 hours after EMR exposure and continued for 30 days.

Exposure system and design

We used six rats in the exposure system in the same time [Fig. 1]. Details of exposure system have been described in detail elsewhere.[16,17] We used power values as 1 mW/m^2 in the experiments as described below. A “SET ELECO” generator from Set Electronic Co, Istanbul (Turkey), provided with a half-wave dipole antenna system was used to irradiate the cells with a 2.45 GHz radio frequency with 217 Hz pulses. The electric field density was set at 11 V/m in order to get a 0.1 W/kg whole-body average specific absorption rate (SAR) and electromagnetic radiation values estimated for 2.45 GHz set up.

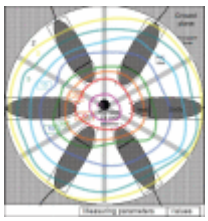

Figure 1
The experimental setup for irradiation of rats[5,6]

The EMR dose was calculated from the measured electric field density (V/m). SAR values were calculated by using electric properties of tissue sample and measured electric field intensities for every distance in certain frequency. These values were shown in Table 1.

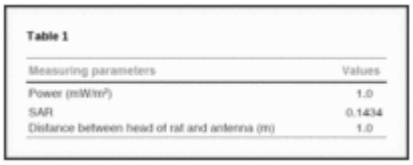

Table 1

Measuring parameters	Values
Power (mW/m²)	1.0
SAR	0.1434
Distance between head of rat and antenna (m)	1.0

Table 1

The rats of Group A2 were placed in the cylindrical restrainer with the radio frequency source switched off during times similar to those used for irradiation. The A1 control animals were kept in their cage without any treatment or restraint of any kind.

Preparation of lens samples

Head of all animals were cut and the lens samples were dissected as described in own previous study.[18] The lens tissues were placed into glass bottles, labeled and stored in a deep freeze (−33°C) until processing (maximum 15 days). After weighing, The lens samples were placed on ice, and homogenized (2 minutes at 5000 rpm) in a five volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), by using a glass ultrasonic homogenizer (HD 2070, Bandelin Ultrasonic homogenizer, Bandelin Electronics GmbH and Co. KG, Berlin, Germany). All preparation procedures were performed on ice. The lens homogenate samples were used for immediate lipid peroxidation, GSH levels and enzyme activities.

Lipid peroxidation determinations

Lipid peroxidation levels in the lens homogenate were spectrophotometrically (at 532 nm) measured with the thiobarbituric-acid reaction by the method of Placer *et al.*[19]

Reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and protein assays

The GSH content in the lens samples was spectrophotometrically measured at 412 nm using the method of Sedlak and Lindsay.[20] Standard curve of the GSH were prepared with different concentrations of GSH. GSH-Px activities of the lens homogenate were measured spectrophotometrically at 37 °C and 412 nm according to the Lawrence and Burk.[21] The protein content in the lens homogenate was measured by method of Lowry *et al.*,[22] with bovine serum albumin as the standard.

Statistical analyses

All results are expressed as means ± standard deviation (SD). To determine the effect of treatment, data were analyzed using analysis of Mann-Whitney U test. *P*values of less than 0.05 were regarded as significant. Data was analyzed using the Statistical Package for the Social Sciences software (SPSS) statistical program (version 17.0 software, SPSS Inc. Chicago, IL, USA).

Go to:

Results and Discussion

In this study, we found firstly in the literature that 2.45 GHz EMR, to which people are intensely Wi-Fi exposed in daily life, increased malondialdehyde (MDA), the indicator of lipid peroxidation and oxidative stress on the lens, and decreased antioxidants including GSH level and GSH-Px activity. Oxidative stress and thus, ROS, are believed to be a principal cause of noncongenital cataract. ROS have been implicated in the development of lens opacification, which has been demonstrated in both experimental animal models[18,23] and in cultured lens systems.[24,25] Cataract patients also exhibited elevated levels of oxidative stress.[18] ROS including superoxide and singlet oxygen can also be generated in the lens and aqueous humor, which explains why areas of the world with higher intensity of EMR irradiation have higher incidence of cataracts.[26]

Levels of lipid peroxidation are shown in [Fig. 2]. Mean values of cage control, sham control, Wi-Fi and Wi-Fi + melatonin groups as μM/g protein were 10.8, 10.7, 11.4 and 9.8, respectively. The lipid peroxidation levels were insignificantly higher in Wi-Fi group than in cage and sham control groups. However, lipid peroxidation levels were significantly ($P < 0.05$) lower in Wi-Fi + melatonin group than in Wi-Fi and control groups.

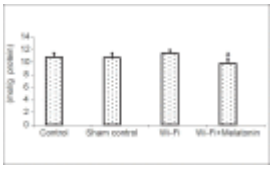

Figure 2
The effects of melatonin on lipid peroxidation levels on lens of Wi-Fi-induced EMR. (Mean ± SD). (mean ± SD).[a]$P < 0.05$ versus Wi-Fi and control groups

Levels of GSH are shown in [Fig. 3]. Mean values of cage control, sham control, Wi-Fi and Wi-Fi + melatonin groups as μM/g protein were 3.08, 3.12, 2.95 and 3.41, respectively. The GSH levels were insignificantly decreased in Wi-Fi group. However, GSH levels were significantly ($P < 0.05$) higher in Wi-Fi + melatonin group than in Wi-Fi group.

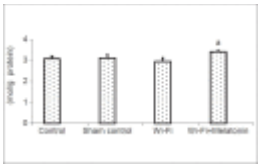

Figure 3
The effects of melatonin on reduced glutathione (GSH) levels on lens of Wi-Fi-induced EMR. (Mean ± SD). (mean ± SD).[a]$P < 0.05$ versus Wi-Fi group

Activities of GSH-Px are shown in [Fig. 4]. Mean values of cage control, sham control, Wi-Fi and Wi-Fi + melatonin groups as IU/g protein were 9.8, 10.4, 8.8 and 11.4, respectively. The GSH-Px activities were significantly ($P < 0.05$) lower in Wi-Fi group than in cage and sham control groups. The antioxidant enzyme activities were modulated by melatonin administration and their activities were significantly higher in Wi-Fi + melatonin group than in Wi-Fi ($P < 0.01$) and control groups ($P < 0.05$).

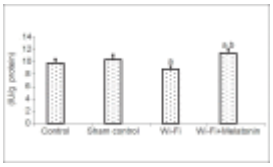

Figure 4

The effects of melatonin on reduced glutathione peroxidase (GSH-Px) activities on lens of 2.45 GHz induced EMR. (Mean ± SD). (mean ± SD).[a]$P < 0.05$ versus controls.[b]$P < 0.01$ versus Wi-Fi group

GSH is the most prevalent low molecular weight thiol containing antioxidant within mammalian cells. The GSH acts to protect cellular constituents from oxidative damage by directly reacting with oxidants or as the substrate for GSH-Px to scavenge peroxides.[27] It is believed that the high content of GSH in the lens protects thiols in structural proteins and enzymes for proper biological functions. The endogenous high level of GSH plays a crucial role as the first line of defense against exogenous and endogenous ROS and enables lens proteins to remain in a reduced state.[27] The ROS are continuously formed and are detoxified by superoxide dismutase, GSH-Px and catalase. Excessive production of free-radicals and the consumption of antioxidants lead to insufficient endogenous defense mechanisms. Lipid peroxidation values as MDA were increased in the lens samples by the Wi-Fi frequency exposure although GSH-Px activity was decreased by the exposure. The GSH-Px activity decreased in the lens samples because it may relate to the used antioxidants due to decrease ROS production and the compensatory in the antioxidant redox system.

Melatonin, as well as its metabolites, act directly to detoxify ROS such as hydroxyl radicals, peroxynitrite anion, singlet oxygen and reactive nitrogen species such as nitric oxide.[2,28] Melatonin possesses an electron-rich aromatic indole ring and acts as an electron-donor, thus having the ability to reduce and repair electrophilic radicals.[28] In addition, melatonin stimulates antioxidant enzymes such as superoxide dismutase and GSH-Px and they exert an antioxidative effect, indirectly.[27] Melatonin, as well as its metabolites, terminates the initiation and propagation of lipid peroxidation.[2] Melatonin is a stabilizer of cell membranes against oxidative stress.[28] In the current study we observed GSH and GSH-Px values were increased by melatonin administration although lipid peroxidation levels decreased due to its antioxidant protector effects and free radical scavenger effects. Consistent with results of the current study, studies using ultraviolet radiation[29] and ionize radiation[30] as the oxidant agent, in which antioxidant efficacy of melatonin on the lens was demonstrated, reported that oxidant-antioxidant indicators were similarly affected.

In conclusion, the results presented for Wi-Fi on lens are not consistent with a generalized antioxidant abnormality although melatonin induced modulator role on the over production of ROS. The beneficial effects of melatonin on antioxidant systems included regulation of the lipid peroxidation, GSH-Px and GSH values in the lens.

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Acknowledgment

Authorship: MN formulated the present hypothesis. MN and LT were responsible for writing the report. SD was responsible for analysis of the data. ΦT and MCK made critical revision for the manuscript.

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Effects of olive leave extract on metabolic disorders and oxidative stress induced by 2.45 GHz WIFI signals.

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Abstract

We investigated the effect of olive leaves extract administration on glucose metabolism and oxidative response in liver and kidneys of rats exposed to radio frequency (RF). The exposure of rats to RF (2.45 GHz, 1h/day during 21 consecutive days) induced a diabetes-like status. Moreover, RF decreased the activities of glutathione peroxidase (GPx, -33.33% and -49.40%) catalase (CAT, -43.39% and -39.62%) and the superoxide dismutase (SOD, -59.29% and -68.53%) and groups thiol amount (-62.68% and -34.85%), respectively in liver and kidneys. Indeed, exposure to RF increased the malondialdehyde (MDA, 29.69% and 51.35%) concentration respectively in liver and kidneys. Olive leaves extract administration (100 mg/kg, ip) in RF-exposed rats prevented glucose metabolism disruption and restored the activities of GPx, CAT and SOD and thiol group amount in liver and kidneys. Moreover, olive leave extract administration was able to bring down the elevated levels of MDA in liver but not in kidneys. Our investigations suggested that RF exposure induced a diabetes-like status through alteration of oxidative response. Olive leaves extract was able to correct glucose metabolism disorder by minimizing oxidative stress induced by RF in rat tissues.

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KEYWORDS:

Metabolism; Olive leaves; Oxidative stress; Radio frequencies

- Abstract

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Effects of low level electromagnetic field exposure at 2.45 GHz on rat cornea.

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Abstract

PURPOSE:

To investigate the effects of low level electromagnetic field (low level-EMF) exposure, as frequently encountered in daily life, on the normal rat cornea using histological and stereological method.

METHODS:

Twenty-two adult male Wistar rats were randomly divided into two groups: Study group (n = 11) and control group (n = 11). Rats in the study group were exposed to 2.45 GHz microwave (MW) radiation (11.96 ± 0.89 V/m), 0.25 W/kg specific absorption rate (SAR) for 2 hours each day for 21 days. The corneal thickness and the anterior epithelium corneal thickness were measured using two different methods.

RESULTS:

Using the histological method, the mean corneal thicknesses in the control and study group were 278.9 ± 54.5 μm, and 272.4 ± 85.6 μm, respectively. There was no statistically significant difference between the groups ($p > 0.05$). The anterior corneal epithelium thickness was 28.1 ± 4.9 μm in the control group and 31.7 ± 5.5 μm in the study group. There were statistically differences between the groups with regard to the thickness of anterior epithelium ($p < 0.05$). In the measurement made by the stereological method, the percentage of the cornea occupied by anterior corneal epithelium was 15.94% in the control group and 17.9% in the study group. Despite the fact that there was a relation between increased anterior epithelial area (AEA) and radiation exposure, no statistically significant relationship in area fraction of each compartment was found between the control and study groups.

CONCLUSIONS:

Results of this preliminary study show that exposure to MW radiation might cause alterations in the rat cornea.

PMID:
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- Abstract

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Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices.

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Abstract

OBJECTIVE:

To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards.

METHODS:

Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities.

RESULTS:

We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$).

CONCLUSIONS:

These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

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Original Article

Effects of Wi-Fi (2.45 GHz) Exposure on Apoptosis, Sperm Parameters and Testicular Histomorphometry in Rats: A Time Course Study

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Abstract

Objective: In today's world, 2.45-GHz radio-frequency radiation (RFR) from industrial, scientific, medical, military and domestic applications is the main part of indoor-outdoor electromagnetic field exposure. Long-term effects of 2.45-GHz Wi-Fi radiation on male reproductive system was not known completely. Therefore, this study aimed to investigate the major cause of male infertility during short- and long-term exposure of Wi-Fi radiation.

Materials and Methods: This is an animal experimental study, which was conducted in the Department of Anatomical Sciences, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, IRAN, from June to August 2014. Three-month-old male Wistar rats (n=27) were exposed to the 2.45 GHz radiation in a chamber with two Wi-Fi antennas on opposite walls. Animals were divided into the three following groups: I. control group (n=9) including healthy animals without any exposure to the antenna, II. 1-hour group (n=9) exposed to the 2.45 GHz Wi-Fi radiation for 1 hour per day during two months and III.7-hour group (n=9) exposed to the 2.45 GHz Wi-Fi radiation for 7 hours per day during 2 months. Sperm parameters, caspase-3 concentrations, histomorphometric changes of testis in addition to the apoptotic indexes were evaluated in the exposed and control animals.

Results: Both 1-hour and 7-hour groups showed a decrease in sperm parameters in a time dependent pattern. In parallel, the number of apoptosis-positive cells and caspase-3 activity increased in the seminiferous tubules of exposed rats. The seminal vesicle weight reduced significantly in both1-hour or 7-hour groups in comparison to the control group.

Conclusion: Regarding to the progressive privilege of 2.45 GHz wireless networks in our environment, we concluded that there should be a major concern regarding the time-dependent exposure of whole-body to the higher frequencies of Wi-Fi networks existing in the vicinity of our living places.

Keywords: Apoptosis, Electromagnetic Radiation, Testis, Spermatogenesis

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Introduction

Electromagnetic radiation (EMR) from different sources, such as microwave ovens, radar, satellite links, wireless communication, frequency modulation (FM) radio and television (TV) transmitters/ antennas, is the main part of indoor- outdoor electromagnetic field exposure spectrum (1, 2). Widespread usage of industrial, scientific, medical, military and domestic applications with 2.45-GHz radio-frequency radiation is inevitable in today's world. As the Wi-Fi technology is low cost and operates in the unlicensed spectrum at 2.40-2.4 GHz, the leakage of Wi-Fi radiation into the environment is unavoidable (3, 4).

It has been suggested that male infertility during the past several decades is related to the direct or indirect exposure to certain environmental factors such as radio-frequency electromagnetic waves (RF-EMW) (5, 6). The effects of 2.45-GHz EMR on reproductive system have already been shown (7-10). Kumar et al. (11) showed 2.45 GHz microwave exposure causes an increase in caspase-3 and creatine kinase activities in the sperm in addition to a decrease in plasma levels of testosterone and melatonin in the exposed rat. *In vitro* study by Avendano et al. (12), focused on the effect of Wi-Fi radiation on the motility reduction and DNA fragmentation of human spermatozoa. The negative effect of Wi-Fi emitting RF-EMW has been also reported on the *ex vivo* human sperm parameters (13), sexual behavior (14) and testis structure of exposed animals (15). It is believed that exposure to EMR can enhance production of reactive oxygen species (ROS) (9, 12, 15-18). An increase in lipid peroxidation levels in addition to a decrease in antioxidant enzymes and vitamin A and E levels (11, 19) can explain some aspects of 2.45-GHz EMR effect on reproductive tissues of male rats. Kim et al. (20) showed that the effect of exposure to 2.45-GHz EMR on proliferation and differentiation of spermatogonia is correlated with serum sex hormone level. In parallel with defect in spermatogenesis process, the negative effects of 2.45-GHz EMR on histopathological changes and apoptosis status of rat testis are inevitable (7). Nowadays 2.45 GHz wireless networks have become much more commonplace in our environment (21). Wireless devices have been widespread used in our living and working environments for longer exposure times than wireless phones which may have an untoward influence on health (2). According to the Bioinitiative Report (http://www.bioinitiative.org/), current safety guidelines for electromagnetic field (EMF) exposure are not sufficient and should be revised based on data from various toxicological tests (22). Due to whole body exposure to the RF-EMR, we tried to analyze potential effects of 2.45 GHz Wi-Fi radiation from a wireless antenna on the reproductive system of freely moving male rats for short- and long-term. Indeed, the consequences of exposure to the emitted radiofrequency waves from Wi-Fi antenna were the major concerns of the present study.

Materials and Methods

Animals

This is an animal experimental study, which was conducted in the Department of Anatomical Sciences, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran, from June to August 2014. Animals, 3-month old Wistar strain rats (n=27), were maintained as national guidelines and protocols approved by the Institutional Animal Ethics Committee (IAEC no.03/028/07).

All experimental protocols were approved by the Ethics Committee of Zanjan University of Medical Sciences, Zanjan, Iran. Healthy adult male albino rats weighing 250 g, were randomly selected and housed under environmentally controlled conditions. The rats were fed with a standard laboratory diet (Pars Dam Co., Tehran, Iran) and clean drinking water *ad libitum*.

Exposure system

The exposure system was a chamber (180 cm×80 cm×70 cm), designed for whole-body exposure of free-moving rats to a Wi-Fi signal. Two Wi-Fi antennas (NanoStation Loco M2, 2.45 GHz, 8.5 dBi, Ubiquiti Networks, Inc. USA) were placed at the center of two sides of the chamber. A previous study applied a restrainer to fix space between antenna and rat (19). Since it was a stressful condition that could probably affect hormonal balance of animals, we tried to assess the effect of radiation on the free moving animals (14, 23).

Animals were divided into three following groups (n=9 per each group): I. control group including healthy animals without any exposure to the antenna, II. 1-hour group including animals exposed to the 2.45 GHz Wi-Fi radiation one hour per day during two months (1 hour/day/2 months) (7, 14, 20) and III. 7-hour group including animals exposed to the 2.45 GHz Wi-Fi radiation seven hours per day during two months (7 hours/day/2 months). All exposure conditions were coded and analyzed in a blind manner.

Laboratory studies, body and reproductive organ weights

Animals were anesthetized intraperitoneally with a mixture of ketamine (45 mg⁄kg, Sigma- Aldrich, Germany) and xylazine (35 mg⁄kg, Sigma Aldrich, Germany). The weight gain of animal in each group was defined as the differences between initial and final body weights. The reproductive organs including testes, epididymis, seminal vesicles and ventral prostate were accurately weighed following being dissected out from surrounded adipose and connective tissues by an expert anatomist. The relative weights of each dissected reproductive organ were expressed as the weight of organ to the body weight ratio. The samples of testicular tissues were fixed in a 4% buffered formaldehyde solution (Merck, Germany) and then were embedded in paraffin wax (Merck, Germany) using standard techniques for preparing 5-µm thick sections. Other side testicle was randomly dissected out and transferred to a cryotube for storing in liquid nitrogen in order to determine the caspase-3 activity.

Sperm characteristics

Caudal part of epididymis was dissected out and chopped in the 5 ml of Ham's F10 medium solution (GIBCO, USA). Epididymal sperm were collected following 5 minutes incubation at 37˚C to allow sperm to swim out of the epididymal tubules. One drop of sperm suspension was placed on a microscope slide and cover slipped. At least 10 microscopic fields were observed at ×40 magnification by a phase contrast microscope (Olympus BX51, Tokyo, Japan). The sperm motility parameters were recorded according to the World Health Organization (WHO) recommendations. The percentages of progressive, motile, and immotile sperm were expressed as the ratio to the total counted sperm. The sperm count parameters were also obtained by the method described in the WHO recommendations (24). Briefly, 5 µl aliquot of epididymal sperm was diluted with 95 µl of diluents (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue, Merck, Germany), and approximately 10 µl of this diluted specimen was transferred to the counting chambers of the haemocytometer. The cells were counted with a light microscope at ×40 magnification. For morphological abnormalities, sperm smears were drawn on slides and allowed to air-dry overnight. Slides were stained with 1% eosin-Y⁄5% nigrosin (Merck, Germany) and examined at ×40 magnification. Amorphous, hook less, bicephalic, coiled or abnormal tails were considered as the morphological abnormalities (25). The total percentages of abnormal and normal sperm were then calculated.

Histopathological evaluation of spermatogenesis

Either the number of germinal cell layers or Johnson's score were measured for categorizing spermatogenesis in the testes. According to Miller et al. (26) description, the number of germinal epithelial layers was counted in 10 seminiferous tubules. Based on Johnson's method, a score of 1-10 was applied for each cross-sectioned tubule (27).

Apoptosis in reproductive tissues of rats

Germ cell apoptosis was evaluated by terminal deoxynucleotidyl transferase (TdT) enzymemediated dUTP nick end labeling (TUNEL) assay kit (Roche, Germany). Briefly, 5-µm thick paraffin-embedded sections were microwave-pretreated in 10 mM citrate buffer (Merck, Germany, pH=6.0) for 10 minutes. Sections were incubated with blocking solution (3% H_2O_2 in methanol, Merck, Germany) for 10 minutes, then were washed with phosphate-buffered saline (PBS, Merck, Germany). The specimens were incubated with TUNEL reaction mixture (TdT and nucleotide mixtures in reaction buffer) at 37˚C for 60 minutes. Finally the slides were stained with converter-POD (anti-fluorescein antibody, Fab fragment from sheep, conjugated with horse-radish peroxidase-POD) for 30 minutes.

The 3, 3′- Diaminobenzidine (DAB) substrate (Roche, Germany) was applied for color development. TUNEL positive cells exhibited a brown nuclear stain. In each group, the number of stained cells was counted in 100 seminiferous tubules. The number of stained germ cells was counted. Apoptotic index-1 (AI-1) was defined as the number of apoptotic TUNEL-positive cells per 100 tubules and apoptotic index-2 (AI-2) as the number of tubules containing apoptotic cells per 100 tubules. All of measurements were performed by an expert technician who was blinded to the experi-

ment procedure.

Caspase-3 activity assay

Briefly, lysis buffer at pH=7.5, including 10 mM Tris-HCL, 10 mM NaH_2PO_4/$NaHPO_4$, 130 mM NaCl, 1% Triton-X100 and 10 mM NaPPi, all materials were purchased from Merck products-Germany that were added to the testes tissue samples and lysates were incubated at 4˚C for 20 minutes. The lysates were centrifuged at 14000 rpm and stored in liquid nitrogen for further analysis. Next 100 ml proteins from lysates were incubated with Ac-DEVD-pNA in a 96-well plate at 37˚C for 1 hour, and colorimetric substrate (DEVD-AFC, Biomol, Plymouth Meeting, PA, USA) was preferentially cleaved by caspase- 3. The amounts of 7-amino-4-methyl-coumarin (AMC) were monitored 1 hour with a plate reader (Anthos2020, USA) and absorption was measured, normalized to the absorbance of time zero and expressed as percent of control.

The data were expressed as mean ± standard errors of the mean (SEM). The variables were analyzed by one-way ANOVA. When a significance found, Tukey post hoc tests were performed. All analyses were performed using the SPSS (SPSS Inc., Chicago, IL, USA) version 16. The statistical significance level was set at $P \leq 0.05$.

Results

Table 1 shows two months exposure of animals to the 2.45 GHz Wi-Fi radiation in the designed exposure apparatus (Fig.1), indicating no significant changes in the body weight of both 1- and 7-hour groups.

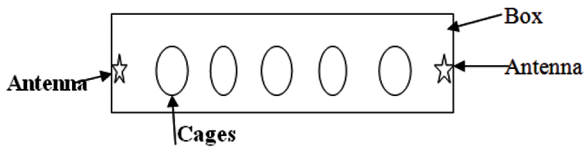

Fig.1: Represents a schematic picture of designed apparatus as the exposure system. Box dimension was 180 cm×80 cm×70 cm. Two Wi-Fi antennas (NanoStation Loco M2, 2.45 GHz, 8.5 dBi, Ubiquiti Networks, Inc. USA) were placed at the center of two sides of the chamber. Animal cages were placed between two antennas.

Despite right and left seminal vesicles, 1 hour and 7 hours chronic exposure caused no significant changes in the relative weight of testicles or other accessory sex organs. The relative weight of both right and left seminal vesicles reduced significantly ($P \leq 0.001$) following two months chronic exposure of animals to the 2.45 GHz Wi-Fi radiation either for 1 hour per day or 7 hours per day (Table 1).

Table 1: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on the weights of testis, epididymis, prostate and seminal vesicle in mature male rats

Groups	Relative right testis weight (%)	Relative left testis weight (%)	Relative right epididymis weight (%)	Relative left epididymis weight (%)	Relative right Seminal vesicle weight (%)	Relative left Seminal vesicle weight (%)	Relative Ventral prostate weight (%)	Weight gain (g)
Control	0.43 ± 0.01	0.45 ± 0.01	0.19 ± 0.0	0.18 ± 0.0	0.18 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	59.37 ± 3.83
1-hour group	0.42 ± 0.01	0.43 ± 0.01	0.18 ± 0.0	0.18 ± 0.0	0.08 ± 0.0[1]	0.08 ± 0.01[1]	0.19 ± 0.0	40.11 ± 8.90
7-hour group	0.41 ± 0.01	0.42 ± 0.01	0.17 ± 0.0	0.17 ± 0.0	0.08 ± 0.02[1]	0.09 ± 0.0[1]	0.22 ± 0.01	50.54 ± 6.88

Values are expressed as mean ± standard errors of the mean (SEM). Chronic exposure to the 2.45 GHz Wi-Fi radiation caused significant differences in the relative weights of both right and left seminal vesicles. The right and left seminal vesicles column were compared with the control group. 1-hour group; exposed to the 2.45 GHz Wi-Fi radiation one hour per day during two months (1 hour/day/2 months). 7-hour group; exposed to the 2.45 GHz Wi-Fi radiation seven hour per day during two months (7 hour/day/2 months).[1]; P value≤0.001.

We examined the proportion of the different sperm motility grades as shown in figure 2. Two months exposure to the 2.45 GHz Wi-Fi radiation caused significant changes on the sperm motility parameters (Fig.2). Although the percentage of progressive sperm showed no significant differences in the experimental groups, the percentages of total motility parameters, considered as the percentage of progressive and motile sperm, reduced significantly in both 1- and 7-hour groups. Therefore, our findings showed a significant reduction in the percentage of motile sperm in 1-hour (27.75 ± 1.27 vs. 44.89 ± 0.81, P≤0.001) and 7-hour (31.87 ± 1.58 vs. 44.89 ± 0.81, P≤0.001) groups as compared to control group.

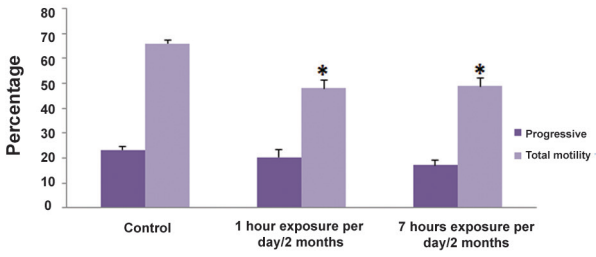

Fig.2: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on total percentage of progressively motile sperm. Values are expressed as mean ± standard errors of the mean (SEM). *; P value≤0.001.

Table 2 shows that chronic exposure to the 2.45 GHz Wi-Fi radiations showed a clear negative impact on the concentration parameters. Sperm samples from both 1-hour (P≤0.001) and 7-hour groups (P≤0.05) exhibited a significant lower concentration as compared to the control group. In parallel with the sperm count reduction, the proportion of normal to abnormal sperm showed a similar reduction in the both 1- and 7-hour groups.

Table 2: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiations on the concentration parameters

	Control	1-hour group	7-hour group
Sperm count (×10⁶/ml)	3.49 ± 1.72	2.16 ± 2.77[2]	2.68 ± 1.261
Normal sperm (%)	87.34 ± 1.05	78.66 ± 1.22[2]	83.68 ± 0.901

Values are expressed as mean ± standard errors of the mean (SEM). The 1-hour and 7-hour groups were compared to the control ones. 1-hour group: exposed to the 2.45 GHz Wi-Fi radiation one hour per day during two months (1 hour/day/2 months). 7-hour group: exposed to the 2.45 GHz Wi-Fi radiation seven hour per day during two months (7 hour/day/2 months). [1]; P≤0.05 and [2]; P≤0.001.

Table 3 shows that the 1-hour group exposed to the 2.45 GHz Wi-Fi radiations demonstrated a normal architecture of the seminiferous tubules and interstitial tissue. The germinal epithelium of testis was intact with an average thickness of about five cell layers. On the contrary, 7-hour group exposed to the 2.45 GHz Wi-Fi radiations caused a significant decrease in both the number of germ cell layers (P≤0.01) and the mean testicular score (P≤0.001). Quantitative and descriptive analysis of TUNEL stained slides in figure 3A and B respectively, show that in parallel with the significant reduction in both the number of germ cell layers and the Johnson's criteria of the 7-hour group, evaluation of apoptotic indexes showed a significant increase in the either the number of apoptotic cells (P≤0.001) or positive tubules per 100 tubules (P≤0.001) in the same group. As it is shown in the figure 4, the increased level of caspase-3 can be a good explanation for testicular apoptosis occurring in the testis of 7-hour animals. Interestingly, lack of significant differences in the number of germ cell layers and the mean testicular score of 1-hour group was accompanied with lack of significant criteria in apoptotic indexes and the caspase-3 concentration. However, two experimental groups showed a significant differences in apoptotic indexes, caspase 3 activity and Johnson's criteria.

Table 3: The Effects of chronic exposure to the 2.45 GHz Wi-Fi radiations on apoptosis of spermatogenesis

	Johnson's criteria	Number of germ cell layers
Control	9.48 ± 0.14	5.58 ± 0.08
1-hour group	9.24 ± 0.05	5.61 ± 0.05
7-hour group	8.75 ± 0.06[2]*	5.25 ± 0.05[1]*

Values are expressed as mean ± standard errors of the mean (SEM). The 1-hour and 7-hour groups were compared to the control ones. [1]; P≤0.01, [2]; P≤0.001 and *; The comparison between 1-hour and 7-hour groups. 1-hour group: exposed to the 2.45 GHz Wi-Fi radiation one hour per day during two months (1 hour/day/2 months). 7-hour group: exposed to the 2.45 GHz Wi-Fi radiation seven hour per day during two months (7 hour/day/2 months). P≤0.001.

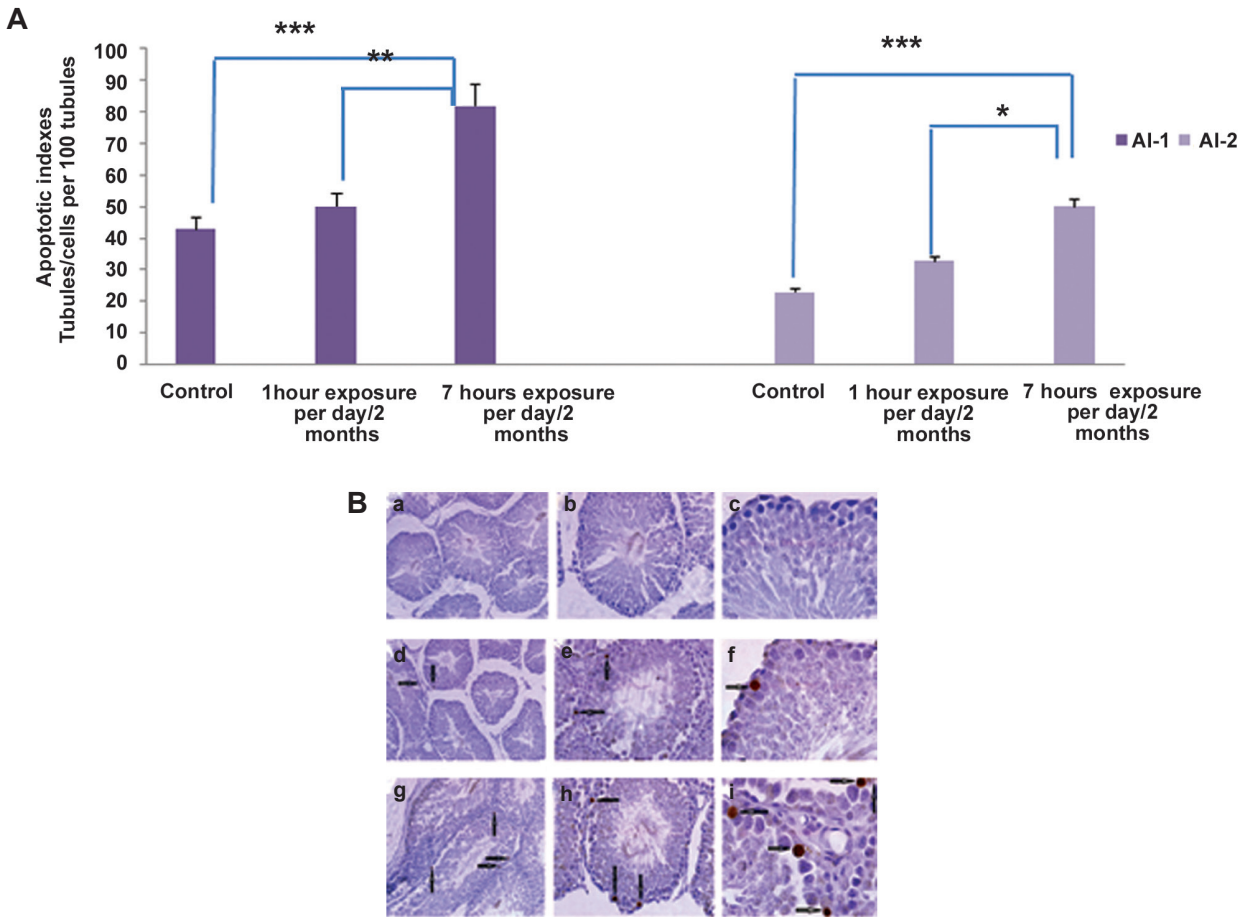

Fig.3: A. The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on the apoptotic indexes as either number of apoptotic terminal deoxynucleotidyl transferase (TdT) enzyme mediated dUTP nick end labeling (TUNEL)-positive cells per 100 tubules (AI-1) or the number of tubules containing apoptotic cells per 100 tubules (AI-2). Values are expressed as mean ± standard errors of the mean (SEM). The 1-hour and 7-hour groups were compared to the control ones. ***; P≤0.001, **; P≤0.01 and *; P≤0.05 and **B.** The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on the apoptosis of spermatogenic cell line. Control group (**a.** magnification ×10, **b.** magnification ×40 and **c.** magnification ×100). 1-hour group (**d.** magnification ×10, **e.** magnification ×40 and **f.** magnification ×100). 7-hour group (**g.** magnification ×10, **h.** magnification ×40 and **i.** magnification ×100). (scale bar=100 μm) arrows show apoptotic cells.

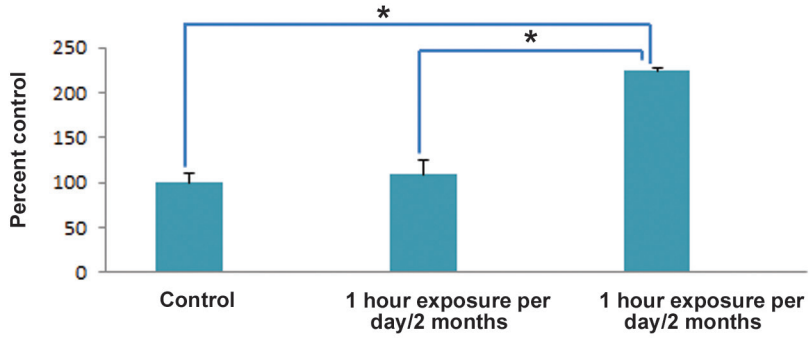

Fig.4: The effect of chronic exposure to the 2.45 GHz Wi-Fi radiation on the concentration of caspase-3. Values are expressed as mean ± standard errors of the mean (SEM). *; P≤0.001.

Discussion

Decline in male fertility, as one of parameters in this study, is considered as a major concern during the past several decades. It has been suggested that direct or indirect exposure to RF-EMW as the main environmental factor plays a dominant role in the observed decline (28). The 2400-2500 GHz radio frequency emitting from Wi-Fi-enabled devices has a long exposure time over a very wide area (2, 19, 21). Hence, this transmitted energy can be absorbed by human body (8, 29).

No deleterious effects of 2.4 GHz Wi-Fi exposure on the body weight and reproductive organ weights were observed in the either 1- or 7-hour groups; however, exposure effect on the seminal vesicle weights was observed. This present result is in line with previous reported animal experiment that demonstrated no adverse effects of 2.45 GHz radio-frequency exposure on the body weight (14) as well as testis and prostate weights (15, 19). Interestingly, 1 hour and 7 hours exposure caused a decline in seminal vesicles weight in comparison to related value of the control group. Although there is no previous report indicating the deleterious effect of 2.45 GHz radiation on seminal vesicles, khaki et al. (30) showed that 50 Hz non-ionizing radiation during two months caused a decrease in seminal vesicles weight. It is noted that epithelial cell proliferation in the seminal vesicles is testosterone-dependent (31). It has been shown that RF-EMF exposure probably reduces the serum testosterone in experimental animals (32, 33).

Alternatively, deficiency in blood testosterone can alter epithelial proliferation in the seminal vesicles. Specifically, Kumar et al. (11) showed that long-term exposure of 2.45 GHz radiation from microwave source can reduce the level of serum testosterone in rats. Consequently, we speculated that the reduced seminal vesicle weight following 2.45 GHz exposure is likely to be related to the reduction of serum testosterone in rats.

Some evidences have indicated that sperm abnormalities are frequent following exposure to RF-EMW (34, 35). We found that sperm concentration, motility and morphology were affected significantly by exposure to the 2.45 GHz RFR from a Wi-Fi antenna. The observed effects were dependent on the longevity of exposure per day. Recent *in vitro* pilot studies on the effect of exposure of the 2.45 GHz RFR on human ejaculated semen found changes in the motility and DNA fragmentation of exposed sperm (12, 13). Kim et al. (20) found no significant reduction in the epididymal sperm count after exposure of rats to the 2.45 GHz EMF [a designed magnetron (Samsungelectronics, Korea) operating at 2.45 GHz by Institute of Biomedical Engineering, Yeungnam University, Daegu, Korea] for 1 hour or 2 hours per day during 8 weeks. Moreover, they reported no abnormal morphology in the exposed groups.

It was also shown that microwave radiation decreases the sperm count (20). A plausible explanation for the impaired sperm motility could be induced oxidative stress by RF-EMW from Wi-Fi devices (12). Oxidation of phospholipids, as a major component in the sperm mitochondrial sheath (36), can disturb mitochondrial membrane potential which causes high levels of ROS to be released into the cytoplasm, leading to deplete the energy supply and to affect both sperm motility and kinetics (37, 38). Peroxidation of unesterified polyunsaturated fatty acids in the cell membrane of spermatozoa can lead to cell death as well (39). However, an *in vitro* pilot study by Oni et al. (13) showed that 1 hour exposure of 2.45 RFR from a laptop antenna (a 2.4 GHz picostation by Ubiquity Networks, USA) had no effects on sperm concentration and sperm head, whereas tail and middle piece defect were evitable following exposure to the RFR. The negative effect of chronic RF exposure from cell phones on the count and the quality of sperm was also reported in the previous researches (40, 41). Interestingly, the negative correlation between both abnormal structure and decreased motility of sperm with the longevity of exposure to the RFR from mobile phones was showed by Wdowiak et al. (42). It is believed that EMF, especially extremely low frequency, induces free radical production that is responsible for sperm deformities (43). Although, the mechanism of cascade is unknown, it has been recently demonstrated that depletion in the activity of both histone kinase and protein kinase may serve as a measure of microwave EMF's ability to affect spermatogenesis

and cell cycle in sperm (8).

In the testis tissue of the animals exposed to 7 hours of 2.45 GHz Wi-Fi radiation for 60 days, the number of germinal cell layers (5.25 ± 0.05 vs. 5.58 ± 0.08, P≤0.01) and Johnson's score (8.75 ± 0.06 vs. 9.48 ± 0.14, P≤0.001) showed a significant reduction as compared to control group. In parallel, the profound DNA damage in 7-hour group was accompanied with an increase in the activity of caspase-3. In accordance with these findings, several authors focused mainly on the destructive effects of RFR on the germinal cell layers of male reproductive organ (11, 14, 15, 19-20, 32, 34, 41). It is shown that 2.45 GHz microwave radiation decreases the diameters of seminiferous tubule (41, 44). Saygin et al. (7) showed changes in histopathology and apoptosis status of rat testis under exposure to 2.45-GHz EMF, at 3.21 W/kg specific absorption rate for 60 minutes/day for 28 days.

On the other hand, Poulletier de Gannes et al. (14) found no microscopic lesions in the testes of male Wistar rats by exposing animals to the 2450 MHz Wi-Fi signal (1 hour/day, 6 days/week, 0.08 and 4 specific absorption rate). Moreover, Kim et al. (20) showed that both the measured diameter of seminiferous tubule and average Johnson's score of testicular biopsy did not change significantly by exposure to the 2.45 GHz EMF (1 hour or 2 hours per day/8 weeks, 1.41W/Kg and 60.1 mV/m electric field intensity. Although they observed no significant difference in the number of spermatids, a significant difference was seen in the number of spermatocytes between the control and exposed group. Atasoy et al. (15) applied standard wireless gateways (2.437 GHz, 24 hours a day for 20 weeks) and their results showed that median values of testicular biopsy score, using Johnson's scale, were significantly lower in the exposed than the control group. They attributed the occurrence of DNA damage to the decreased levels of catalase and glutathione peroxidase activity as a consequence of 2.45 GHz RF that led to induce oxidative stress. Apoptosis is induced by ROS through cytochrome C and caspases-3 and -9 which in turn leads to a high rate of single and double DNA strand break (45). Actually, caspase-3 is a key mediator of apoptosis (46).

It is showed that 2.45-GHz microwave exposure (2 hours per day/ 2 months) increases caspase and creatine kinase activities and decreases melatonin level in the testes of exposed rats (11). The role of 2.45-GHz EMF in inducing oxidative stress by enhancing the lipid peroxidation, free radical formation and modifying antioxidant systems has been approved previously (19, 47, 48). Interestingly, the 2.45 GHz induced oxidative stress was attributed to the reduced levels of testosterone and non-enzymatic antioxidants such as vitamin A and E (19, 32).

Conclusion

High frequency, specifically 2.45 GHz Wi-Fi radiation, induces a decrease in sperm parameters along with an increase in apoptosis-positive cells and caspase-3 activity in the seminiferous tubules of Wistar rats, specially in 7-hour group. It reduced seminal vesicle weight following 2.45 GHz exposure. Considering the progressive privilege of 2.45 GHz wireless networks in our environment, we concluded that there should be a major concern about the time-dependent exposure of our body to the higher frequencies of Wi-Fi antenna.

Acknowledgments

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- Abstract

Metab Brain Dis. 2014 Sep;29(3):787-99. doi: 10.1007/s11011-014-9549-9. Epub 2014 May 3.

Electromagnetic radiation (Wi-Fi) and epilepsy induce calcium entry and apoptosis through activation of TRPV1 channel in hippocampus and dorsal root ganglion of rats.

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Abstract

Incidence rates of epilepsy and use of Wi-Fi worldwide have been increasing. TRPV1 is a Ca(2+) permeable and non-selective channel, gated by noxious heat, oxidative stress and capsaicin (CAP). The hyperthermia and oxidant effects of Wi-Fi may induce apoptosis and Ca(2+) entry through activation of TRPV1 channel in epilepsy. Therefore, we tested the effects of Wi-Fi (2.45 GHz) exposure on Ca(2+) influx, oxidative stress and apoptosis through TRPV1 channel in the murine dorsal root ganglion (DRG) and hippocampus of pentylentetrazol (PTZ)-induced epileptic rats. Rats in the present study were divided into two groups as controls and PTZ. The PTZ groups were divided into two subgroups namely PTZ + Wi-Fi and PTZ + Wi-Fi + capsazepine (CPZ). The hippocampal and DRG neurons were freshly isolated from the rats. The DRG and hippocampus in PTZ + Wi-Fi and PTZ + Wi-Fi + CPZ groups were exposed to Wi-Fi for 1 hour before CAP stimulation. The cytosolic free Ca(2+), reactive oxygen species production, apoptosis, mitochondrial membrane depolarization, caspase-3 and -9 values in hippocampus were higher in the PTZ group than in the control although cell viability values decreased. The Wi-Fi exposure induced additional effects on the cytosolic Ca(2+) increase. However, pretreatment of the neurons with CPZ, results in a protection against epilepsy-induced Ca(2+) influx, apoptosis and oxidative damages. In results of whole cell patch-clamp experiments, treatment of DRG with Ca(2+) channel antagonists [thapsigargin, verapamil + diltiazem, 2-APB, MK-801] indicated that Wi-Fi exposure induced Ca(2+) influx via the TRPV1 channels. In conclusion, epilepsy and Wi-Fi in our experimental model is involved in Ca(2+) influx and oxidative stress-induced hippocampal and DRG death through activation of TRPV1 channels, and negative modulation of this channel activity by CPZ pretreatment may account for the neuroprotective activity against oxidative stress.

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EMF radiation at 2450 MHz triggers changes in the morphology and expression of heat shock proteins and glucocorticoid receptors in rat thymus.

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Abstract

AIMS:

Electromagnetic fields (EMFs) can act as inducers or mediators of stress response through the production of heat shock proteins (HSPs) that modulate immune response and thymus functions. In this study, we analyzed cellular stress levels in rat thymus after exposure of the rats to a 2.45 GHz radio frequency (RF) using an experimental diathermic model in a Gigahertz Transverse Electromagnetic (GTEM) chamber.

MAIN METHODS:

In this experiment, we used H&E staining, the ELISA test and immunohistochemistry to examine Hsp70 and Hsp90 expression in the thymus and glucocorticoid receptors (GR) of 64 female Sprague–Dawley rats exposed individually to 2.45 GHz (at 0, 1.5, 3.0 or 12.0 W power). The 1 g averaged peak and mean SAR values in the thymus and whole body of each rat to ensure that sub-thermal levels of radiation were being reached.

KEY FINDINGS:

The thymus tissue presented several morphological changes, including increased distribution of blood vessels along with the appearance of red blood cells and hemorrhagic reticuloepithelial cells. Levels of Hsp90 decreased in the thymus when animals were exposed to the highest power level (12 W), but only one group did not show recovery after 24 h. Hsp70 presented no significant modifications in any of the groups. The glucocorticoid receptors presented greater immunomarking on the thymic cortex in exposed animals.

SIGNIFICANCE:

Our results indicate that non-ionizing sub-thermal radiation causes changes in the endothelial permeability and vascularization of the thymus, and is a tissue-modulating agent for Hsp90 and GR.

KEYWORDS:

GTEM; HSP; Non-ionizing radiation; SAR; Sub-thermal; Thymus

Exp Biol Med (Maywood). 2015 Sep;240(9):1123-35. doi: 10.1177/1535370214567611. Epub 2015 Feb 2.

Exposure to non-ionizing radiation provokes changes in rat thyroid morphology and expression of HSP-90.

Misa-Agustiño MJ[1], Jorge-Mora T[1], Jorge-Barreiro FJ[1], Suarez-Quintanilla J[1], Moreno-Piquero E[2], Ares-Pena FJ[2], López-Martín E[3].

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Abstract

Non-ionizing radiation at 2.45 GHz may modify the morphology and expression of genes that codify heat shock proteins (HSP) in the thyroid gland. Diathermy is the therapeutic application of non-ionizing radiation to humans for its beneficial effects in rheumatological and musculo-skeletal pain processes. We used a diathermy model on laboratory rats subjected to maximum exposure in the left front leg, in order to study the effects of radiation on the nearby thyroid tissue. Fifty-six rats were individually exposed once or repeatedly (10 times in two weeks) for 30 min to 2.45 GHz radiation in a commercial chamber at different non-thermal specific absorption rates (SARs), which were calculated using the finite difference time domain technique. We used immunohistochemistry methods to study the expression of HSP-90 and morphological changes in thyroid gland tissues. Ninety minutes after radiation with the highest SAR, the central and peripheral follicles presented increased size and the thickness of the peripheral septa had decreased. Twenty-four hours after radiation, only peripheral follicles radiated at 12 W were found to be smaller. Peripheral follicles increased in size with repeated exposure at 3 W power. Morphological changes in the thyroid tissue may indicate a glandular response to acute or repeated stress from radiation in the hypothalamic-pituitary-thyroid axis. Further research is needed to determine if the effect of this physical agent over time may cause disease in the human thyroid gland.

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KEYWORDS:

Gigahertz; Thyroid; follicle; heat shock protein; septa; specific absorption rate

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Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic.

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Abstract

PURPOSE:

To investigate the oxidative damage and protective effect of garlic on rats exposed to low level of electromagnetic fields (EMF) at 2.45 GHz Microwave radiation (MWR).

METHODS:

Thirty-six Wistar rats were divided into three groups. Group I was the control group and not exposed to EMF. Group II and III were exposed to low level EMF (3.68 ± 0.36 V/m) at 2.45 GHz MWR for 1 hour/day for 30 consecutive days. Daily 500 mg/kg garlic was given to Group III during the study period. At the end of the study, thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and 8-hydroxydeoxyguanosine (8-OHdG) levels were investigated in brain tissue and blood samples.

RESULTS:

Exposure to low level of EMF increased 8-OHdG level in both plasma and brain tissue whereas it increased AOPP level only in plasma. Garlic prevented the increase of 8-OHdG level in brain tissue and plasma AOPP levels.

CONCLUSIONS:

It may be concluded that low level EMF at 2.45 GHz MWR increases the DNA damage in both brain tissues and plasma of the rats whereas it increases protein oxidation only in plasma. It may also be argued that the use of garlic decreases these effects.

KEYWORDS:

Antioxidants; E-M fields; oxidative stress

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Investigation of the effects of distance from sources on apoptosis, oxidative stress and cytosolic calcium accumulation via TRPV1 channels induced by mobile phones and Wi-Fi in breast cancer cells.

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Abstract

TRPV1 is a Ca2+ permeable channel and gated by noxious heat, oxidative stress and capsaicin (CAP). Some reports have indicated that non-ionized electromagnetic radiation (EMR)-induces heat and oxidative stress effects. We aimed to investigate the effects of distance from sources on calcium signaling, cytosolic ROS production, cell viability, apoptosis, plus caspase-3 and -9 values induced by mobile phones and Wi-Fi in breast cancer cells MCF-7 human breast cancer cell lines were divided into A, B, C and D groups as control, 900, 1800 and 2450 MHz groups, respectively. Cells in Group A were used as control and were kept in cell culture conditions without EMR exposure. Groups B, C and D were exposed to the EMR frequencies at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) for 1h before CAP stimulation. The cytosolic ROS production, Ca2+ concentrations, apoptosis, caspase-3 and caspase-9 values were higher in groups B, C and D than in A group at 0 cm, 1 cm and 5 cm distances although cell viability (MTT) values were increased by the distances. There was no statistically significant difference in the values between control, 20 and 25 cm. Wi-Fi and mobile phone EMR placed within 10 cm of the cells induced excessive oxidative responses and apoptosis via TRPV1-induced cytosolic Ca2+ accumulation in the cancer cells. Using cell phones and Wi-Fi sources which are farther away than 10 cm may provide useful protection against oxidative stress, apoptosis and overload of intracellular Ca2+. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

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KEYWORDS:

Apoptosis; Breast cancer; Calcium signaling; Mobile phone; Oxidative stress; Wireless internet (Wi-Fi)

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Klebsiella pneumonia, a Microorganism that Approves the Non-linear Responses to Antibiotics and Window Theory after Exposure to Wi-Fi 2.4 GHz Electromagnetic Radiofrequency Radiation

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Go to:

Abstract

Background

Drug resistance is widely believed to be an increasingly serious threat to global public health. We have previously reported that short term exposure of microorganisms to diagnostic ultrasound waves could significantly alter their sensitivity to antibiotics. In our previous studies, *Klebsiella pneumoniae* showed major differences in the sensitivity to antibiotics in exposed and non-exposed samples. This study was aimed at investigating the alteration of antibiotic resistance of *Klebsiella pneumonia*, after exposure to Wi-Fi 2.4 GHz electromagnetic radiofrequency radiation.

Materials and Methods

In this *in vitro* study, three replicate agar plates were used for each test. The antibiotic susceptibility test was carried out using disc diffusion method on Mueller Hinton agar plates and the inhibition zones in both control and exposed groups were measured. A common Wi-Fi router was used in this study as the radiofrequency exposure source. Irradiated samples were exposed to Wi-Fi radiofrequency radiation for 3, 4.5 and 8 hours.

Results

Statistically significant variations of sensitivity to antibiotics were found for all studied antibiotics after 4.5 hours of RF exposure, compared to non-exposed bacteria. Interestingly, the mean diameters of the inhibition zones after 3 hours of exposure were less than those

exposed for 4.5 hours. Following this rise in the sensitivity to antibiotics, a fall was observed in the bacteria exposed for 8 hours for all studied antibiotics.

Conclusion

The findings of this study show a statistically significant rise in the sensitivity of *Klebsiella pneumoniae* to different antibiotics after 4.5 hours of exposure to 2.4 GHz Wi-Fi radiation, followed by a fall after 8 hours of exposure. These observations can be interpreted by the concept of non-linearity in the responses of *Klebsiella pneumoniae* to different antibiotics after exposure to electromagnetic radiofrequency radiation. As in this study a minimum level of effect was needed for the induction of adaptive response, these results also confirm the validity of the so-called "window theory".

Keywords: *Klebsiella*, Non-linearity, Dose Response, Adaptive Response, Antibiogram
Go to:

Introduction

The genus *Klebsiella*, a member of the family Enterobacteriaceae, are nonmotile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule which frequently cause human nosocomial infections including urinary tract, intraabdominal and upper respiratory tract infections (nosocomial pneumonia). Respiratory tract infections caused by *Klebsiella pneumoniae* couple with high rates of mortality and morbidity. Due to increasing frequency rate of strains which are resistant to multiple antimicrobial agents, management of these infections is a challenging issue in microbiology.

Furthermore *K. pneumoniae* is a potential community-acquired pathogen. The current hypothesis is based on this fact that these bacteria acquire multidrug resistance (MDR) through horizontal transfer from antimicrobial resistance genes. Several genes are involved in MDR pattern to commonly antimicrobial agents. Although, high prevalence of drug resistance has been reported in MDR *K. pneumoniae* strains, there is limited information about the genomic features which can be responsible for the high-level of resistance.

We and other investigators have previously reported that radiofrequency radiation can induce adaptive response phenomena [1-6]. We have previously shown that the dose window theory that is well discussed for adaptive responses induced by ionizing radiation is also valid for non-ionizing radiation [7]. As discussed by RE Mitchel, "the adaptive response in mammalian cells and mammals operates within a certain window that can be defined by upper and lower dose thresholds, typically between about 1 and 100 mGy for a single low dose rate exposure" [8]. On the other hand, as indicated by investigators who worked on ionizing radiation-induced adaptive responses [9-11], some of the findings on RF pre-exposures support this theory that the induction of adaptive response requires a minimum level of damage to trigger this phenomenon [7, 12]. In this light, we have reported that there are similar patterns for induction of adaptive response by ionizing and non-ionizing radiations.

Over the past years, our laboratory has focused on studying the health effects of exposure of laboratory animals and human to some common sources of electromagnetic fields such as mobile phones [4, 13-18] and their base stations [19], laptop computers [20], and MRI [21],

as well as occupational exposure to electromagnetic fields generated by cavitrons [17] or radar [22]. On the other hand, over the past several years we have developed techniques for changing the sensitivity of bacteria to antibiotics, heat and UV using physical stressors. Recently, we have shown that short term exposure of bacteria to mechanical waves generated by diagnostic ultrasonic devices could significantly alter their sensitivity to antibiotics. In this paper we present our findings on non-linear responses and window theory in hormetic responses of *K. pneumoniae* to antibiotics after exposure to electromagnetic radiofrequency radiation.

Go to:

Material And Methods

Isolation and identification of isolates

This *in vitro* case control study was performed at the Ionizing and Non-ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences (SUMS), Shiraz, Iran in 2015. The bacterial strains were obtained from the Pasteur Institute of Iran (*Klebsiella pneumonia ATCC 700603*).The samples were cultured on blood agar and MacConkey agar was used for the isolation of microorganism. The culture plates were incubated at 35°C for 18-24 hours and observed for the presence or absence of visible bacterial growth.

Antibiotic susceptibility tests

Antimicrobial susceptibility assay of *K. pneumoniae* was carried out using disc diffusion method on Mueller Hinton agar plates (Figure 1). The fresh cultures of *K. pneumonia* were diluted in Mueller Hinton Broth and matched with the 0.5 MacFarlaned turbidity standards to get 1×10^8 CFU/mL as total count. Bacterial suspensions were spread on mueller-hinton agar (Lio, Italy). The antibiotic discs were placed over the lawn and incubated at 35 °C for 18-24 h. The inhibition zone around each an antibiotic disc was measured in millimeter.

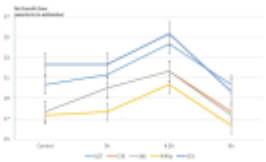

Figure 1
Inhibition zone diameters before and after exposure to Wi-Fi radiofrequency radiation for 3, 4.5 and 8 hours.

Drug susceptibility test was performed for Aztreonam (AZT 30 µg), Cefteriaxone (CTR 30 µg), Imipenem (IMI 10µg), Piperacilline (PIPRA 100 µg), and Cefotaxime (CTX 30µg) for this bacteria. All culture media and antibiotic discs were purchased from ROSCO Diagnostica (DK-2630 Taastrup, Denmark). Results of antibiotic susceptibility assay before and after exposure to Wi-Fi radiofrequency radiation were recorded and analyzed. The inhibition zone of each plate was recorded as the average of two diameters (in mm) measured at right angles to one another. Three replicate agar plates were used for each regime, according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (2013).

Wi-Fi Router

A D-Link Wi-Fi router (D-Link, D-Link Corporation, Taiwan) was used in this study as the RF exposure source. This modem was exchanging data with a laptop computer that was placed in another room (5 meters away from the Wi-Fi router) during the exposure period. The Wi-Fi router operated on power level of 1W and the Specific Absorption Rate at the distance of 5 cm in bacterial suspension (broth medium).

Statistical Methods

The mean diameters of inhibition zones of the 3 replicates in exposed and non-exposed groups were compared using the non-parametric Mann-Whitney test. The significance level was considered at $P < 0.05$.

Go to:

Results

Findings of this study are summarized in Table 1. Compared to the results obtained from unexposed bacteria, statistically significant variations of sensitivity to antibiotics were found for all of the 5 antibiotics after 4.5 hours of RF exposure. As shown in Figure 1 the mean diameters of the inhibition zones either after 3 hours or 8 hours of exposure were less than those of 4.5 hours of exposure.

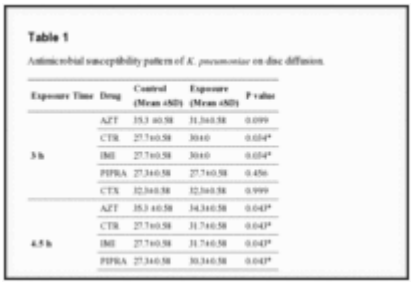

Table 1

Antimicrobial susceptibility pattern of *K. pneumoniae* on disc diffusion.

Exposure Time	Drug	Control (Mean ±SD)	Exposure (Mean ±SD)	P value
3 h	AZT	15.3 ±0.58	31.3±0.58	0.099
	CTR	27.7±0.58	30±0	0.034*
	IMI	27.7±0.58	30±0	0.034*
	PIPRA	27.3±0.58	27.7±0.58	0.456
	CTX	32.3±0.58	32.3±0.58	0.999
4.5 h	AZT	15.3 ±0.58	34.3±0.58	0.043*
	CTR	27.7±0.58	31.7±0.58	0.043*
	IMI	27.7±0.58	31.7±0.58	0.043*
	PIPRA	27.3±0.58	30.3±0.58	0.043*

Table 1

Antimicrobial susceptibility pattern of *K. pneumoniae* on disc diffusion.

Go to:

Discussion

Altogether the findings of this study show a rise in the sensitivity of *Klebsiella* to different antibiotics after 4.5 hours of exposure to 2.4 GHz Wi-Fi radiation, followed by a fall after 8 hours of exposure. These findings can be clearly interpreted by the concept of non-linearity in the responses of these bacteria to antibiotics after exposure to electromagnetic radiofrequency radiation. These results also confirm the validity of the so-called “window theory” in hormetic responses to low levels of either ionizing or non-ionizing radiation. Mitchel has previously stated that the adaptive response in mammalian cells and mammals appears within a certain window with specific upper and lower dose thresholds (typically the upper and lower levels are 1 and 100 mGy, respectively for a single low dose rate exposure) [8]. Furthermore, reserachers who worked on ionizing radiation-induced adaptive responses [9, 11, 23], has shown that the induction of adaptive response requires a minimum level of effect

(damage) to trigger this phenomenon. Mortazavi has recently discussed that there are similar patterns for the induction of adaptive response by ionizing and non-ionizing radiations [7]. As in this study we observed a rise in the diameters of inhibition zone (i.e. sensitivity of *K. pneumoniae* to different antibiotics) after 4.5 hours of exposure to 2.4 GHz Wi-Fi radiation, followed by a fall after 8 hours of exposure, it shows that these bacteria need a minimum level of damage for becoming resistant to antibiotics. It seems that there are specific mechanisms for the induction of bacterial resistance after exposure to radiation. All of the antibiotic discs used in this study played their role through disrupting the cell wall synthesis. Therefore, it can be concluded that permeability of the bacterial cell wall may be affected by radiofrequency radiation. Hence, the entrance and exit of substrates by channels like efflux pumps in the cell wall can be altered by radiation. Based on these findings, we believe that exposure to 2.4 GHz Wi-Fi radiation as a physical method of altering the susceptibility of microorganisms to antibiotics, can open new horizons in challenging fields such as antibiotic therapy of a broad range of diseases.

Go to:

Acknowledgment

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Conflict of Interest: The authors have no potential conflict of interest with regard to the content of this article.

Go to:

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Bioelectromagnetics. 1992;13(6):469-96.

Long-term, low-level microwave irradiation of rats.

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Abstract

Our goal was to investigate effects of long-term exposure to pulsed microwave radiation. The major emphasis was to expose a large sample of experimental animals throughout their lifetimes and to monitor them for effects on general health and longevity. An exposure facility was developed that enabled 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed individually in circularly-polarized waveguides. The exposure facility consisted of two rooms, each containing 50 active waveguides and 50 waveguides for sham (control) exposures. The experimental rats were exposed to 2,450-MHz pulsed microwaves at 800 pps with a 10-microseconds pulse width. The pulsed microwaves were square-wave modulated at 8-Hz. Whole body calorimetry, thermographic analysis, and power-meter analysis indicated that microwaves delivered at 0.144 W to each exposure waveguide resulted in an average specific absorption rate (SAR) that ranged from 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat. Two hundred male, Sprague-Dawley rats were assigned in equal numbers to radiation-exposure and sham-exposure conditions. Exposure began at 8 weeks of age and continued daily, 21.5 h/day, for 25 months. Animals were bled at regular intervals and blood samples were analyzed for serum chemistries, hematological values, protein electrophoretic patterns, thyroxine, and plasma corticosterone levels. In addition to daily measures of body mass, food and water consumption by all animals, O2 consumption and CO2 production were periodically measured in a sub-sample (N = 18) of each group. Activity was assessed in an open-field apparatus at regular intervals throughout the study. After 13 months, 10 rats from each group were euthanized to test for immunological competence and to permit whole-body analysis, as well as gross and histopathological examinations. At the end of 25 months, the survivors (11 sham-exposed and 12 radiation-exposed rats) were euthanized for similar analyses. The other 157 animals were examined histopathologically when they died spontaneously or were terminated in extremis.

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Neurotoxicology. 2015 Dec;51:158-65. doi: 10.1016/j.neuro.2015.10.009. Epub 2015 Oct 25.

Low intensity microwave radiation induced oxidative stress, inflammatory response and DNA damage in rat brain.

Megha K[1], Deshmukh PS[1], Banerjee BD[2], Tripathi AK[1], Ahmed R[1], Abegaonkar MP[3].

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Abstract

Over the past decade people have been constantly exposed to microwave radiation mainly from wireless communication devices used in day to day life. Therefore, the concerns over potential adverse effects of microwave radiation on human health are increasing. Until now no study has been proposed to investigate the underlying causes of genotoxic effects induced by low intensity microwave exposure. Thus, the present study was undertaken to determine the influence of low intensity microwave radiation on oxidative stress, inflammatory response and DNA damage in rat brain. The study was carried out on 24 male Fischer 344 rats, randomly divided into four groups (n=6 in each group): group I consisted of sham exposed (control) rats, group II-IV consisted of rats exposed to microwave radiation at frequencies 900, 1800 and 2450MHz, specific absorption rates (SARs) 0.59, 0.58 and 0.66mW/kg, respectively in gigahertz transverse electromagnetic (GTEM) cell for 60 days (2h/day, 5 days/week). Rats were sacrificed and decapitated to isolate hippocampus at the end of the exposure duration. Low intensity microwave exposure resulted in a frequency dependent significant increase in oxidative stress markers viz. malondialdehyde (MDA), protein carbonyl (PCO) and catalase (CAT) in microwave exposed groups in comparison to sham exposed group ($p<0.05$). Whereas, levels of reduced glutathione (GSH) and superoxide dismutase (SOD) were found significantly decreased in microwave exposed groups ($p<0.05$). A significant increase in levels of pro-inflammatory cytokines (IL-2, IL-6, TNF-α, and IFN-γ) was observed in microwave exposed animal ($p<0.05$). Furthermore, significant DNA damage was also observed in microwave exposed groups as compared to their corresponding values in sham exposed group ($p<0.05$). In conclusion, the present study suggests that low intensity microwave radiation induces oxidative stress, inflammatory response and DNA damage in brain by exerting a frequency dependent effect. The study also indicates that increased oxidative stress and inflammatory response might be the factors involved in DNA damage following low intensity microwave exposure.

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KEYWORDS:

Brain; Cytokines; DNA damage; Microwave radiation; Oxidative stress

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Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue.

Dasdag S[1], Akdag MZ, Erdal ME, Erdal N, Ay OI, Ay ME, Yilmaz SG, Tasdelen B, Yegin K.

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Abstract

PURPOSE:

MicroRNAs (miRNA) play a paramount role in growth, differentiation, proliferation and cell death by suppressing one or more target genes. However, their interaction with radiofrequencies is still unknown. The aim of this study was to investigate the long-term effects of radiofrequency radiation emitted from a Wireless Fidelity (Wi-Fi) system on some of the miRNA in brain tissue.

MATERIALS AND METHODS:

The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (n = 8) and exposure (n = 8). Rats in the exposure group were exposed to 2.4 GHz radiofrequency (RF) radiation for 24 hours a day for 12 months (one year). The same procedure was applied to the rats in the sham group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and their brains were removed. miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p in brain were investigated in detail.

RESULTS:

The results revealed that long-term exposure of 2.4 GHz Wi-Fi radiation can alter expression of some of the miRNAs such as miR-106b-5p (adj p* = 0.010) and miR-107 (adj p* = 0.005). We observed that mir 107 expression is 3.3 times and miR- 106b-5p expression is 3.65 times lower in the exposure group than in the control group. However, miR-9-5p, miR-29a-3p and miR-125a-3p levels in brain were not altered.

CONCLUSION:

Long-term exposure of 2.4 GHz RF may lead to adverse effects such as neurodegenerative diseases originated from the alteration of some miRNA expression and more studies should be devoted to the effects of RF radiation on miRNA expression levels.

KEYWORDS:

2.4 GHz radiofrequency; Wi-Fi; diseases; electromagnetic fields; miRNA in brain

Toxicol Sci. 2015 Dec;148(2):380-99. doi: 10.1093/toxsci/kfv205. Epub 2015 Sep 22.

2.45 GHz Microwave Radiation Impairs Learning and Spatial Memory via Oxidative/Nitrosative Stress Induced p53-Dependent/Independent Hippocampal Apoptosis: Molecular Basis and Underlying Mechanism.

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- [3]*Department of Zoology, Banaras Hindu University, Varanasi 221005, India and cmcbhu@gmail.com.

Abstract

A close association between microwave (MW) radiation exposure and neurobehavioral disorders has been postulated but the direct effects of MW radiation on central nervous system still remains contradictory. This study was performed to understand the effect of short (15 days) and long-term (30 and 60 days) low-level MW radiation exposure on hippocampus with special reference to spatial learning and memory and its underlying mechanism in Swiss strain male mice, Mus musculus. Twelve-weeks old mice were exposed to 2.45 GHz MW radiation (continuous-wave [CW] with overall average power density of 0.0248 mW/cm(2) and overall average whole body specific absorption rate value of 0.0146 W/Kg) for 2 h/day over a period of 15, 30, and 60 days). Spatial learning and memory was monitored by Morris Water Maze. We have checked the alterations in hippocampal oxidative/nitrosative stress, neuronal morphology, and expression of pro-apoptotic proteins (p53 and Bax), inactive executioner Caspase- (pro-Caspase-3), and uncleaved Poly (ADP-ribose) polymerase-1 in the hippocampal subfield neuronal and nonneuronal cells (DG, CA1, CA2, and CA3). We observed that, short-term as well as long-term 2.45 GHz MW radiation exposure increases the oxidative/nitrosative stress leading to enhanced apoptosis in hippocampal subfield neuronal and nonneuronal cells. Present findings also suggest that learning and spatial memory deficit which increases with the increased duration of MW exposure (15 < 30 < 60 days) is correlated with a decrease in hippocampal subfield neuronal arborization and dendritic spines. These findings led us to conclude that exposure to CW MW radiation leads to oxidative/nitrosative stress induced p53-dependent/independent activation of hippocampal neuronal and nonneuronal apoptosis associated with spatial memory loss.

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KEYWORDS:

hippocampal apoptosis; learning and spatial memory; microwave radiation; neurocytoarchitecture; oxidative and nitrosative stress

Plant Signal Behav. 2014;9(4):e28590. doi: 10.4161/psb.28590.

Nanometer-scale elongation rate fluctuations in the Myriophyllum aquaticum (Parrot feather) stem were altered by radio-frequency electromagnetic radiation.

Senavirathna MD[1], Asaeda T, Thilakarathne BL, Kadono H.

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Abstract

The emission of radio-frequency electromagnetic radiation (EMR) by various wireless communication base stations has increased in recent years. While there is wide concern about the effects of EMR on humans and animals, the influence of EMR on plants is not well understood. In this study, we investigated the effect of EMR on the growth dynamics of Myriophyllum aquaticum (Parrot feather) by measuring the nanometric elongation rate fluctuation (NERF) using a statistical interferometry technique. Plants were exposed to 2 GHz EMR at a maximum of 1.42 Wm(-2) for 1 h. After continuous exposure to EMR, M. aquaticum plants exhibited a statistically significant 51 ± 16% reduction in NERF standard deviation. Temperature observations revealed that EMR exposure did not cause dielectric heating of the plants. Therefore, the reduced NERF was due to a non-thermal effect caused by EMR exposure. The alteration in NERF continued for at least 2.5 h after EMR exposure and no significant recovery was found in post-EMR NERF during the experimental period.

KEYWORDS:

electromagnetic radiation; growth dynamics; non-thermal; plant stress; statistical interferometry; wireless communication

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Modulation of wireless (2.45 GHz)-induced oxidative toxicity in laryngotracheal mucosa of rat by melatonin.

Aynali G[1], Nazıroğlu M, Çelik Ö, Doğan M, Yarıktaş M, Yasan H.

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Abstract

It is well known that oxidative stress induces larynx cancer, although antioxidants induce modulator role on etiology of the cancer. It is well known that electromagnetic radiation (EMR) induces oxidative stress in different cell systems. The aim of this study was to investigate the possible protective role of melatonin on oxidative stress induced by Wi-Fi (2.45 GHz) EMR in laryngotracheal mucosa of rat. For this purpose, 32 male rats were equally categorized into four groups, namely controls, sham controls, EMR-exposed rats, EMR-exposed rats treated with melatonin at a dose of 10 mg/kg/day. Except for the controls and sham controls, the animals were exposed to 2.45 GHz radiation during 60 min/day for 28 days. The lipid peroxidation levels were significantly ($p < 0.05$) higher in the radiation-exposed groups than in the control and sham control groups. The lipid peroxidation level in the irradiated animals treated with melatonin was significantly ($p < 0.01$) lower than in those that were only exposed to Wi-Fi radiation. The activity of glutathione peroxidase was lower in the irradiated-only group relative to control and sham control groups but its activity was significantly ($p < 0.05$) increased in the groups treated with melatonin. The reduced glutathione levels in the mucosa of rat did not change in the four groups. There is an apparent protective effect of melatonin on the Wi-Fi-induced oxidative stress in the laryngotracheal mucosa of rats by inhibition of free radical formation and support of the glutathione peroxidase antioxidant system.

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Oxidative stress of brain and liver is increased by Wi-Fi (2.45GHz) exposure of rats during pregnancy and the development of newborns.

Çelik Ö[1], Kahya MC[2], Nazıro•lu M[3].

Author information

Abstract

An excessive production of reactive oxygen substances (ROS) and reduced antioxidant defence systems resulting from electromagnetic radiation (EMR) exposure may lead to oxidative brain and liver damage and degradation of membranes during pregnancy and development of rat pups. We aimed to investigate the effects of Wi-Fi-induced EMR on the brain and liver antioxidant redox systems in the rat during pregnancy and development. Sixteen pregnant rats and their 48 newborns were equally divided into control and EMR groups. The EMR groups were exposed to 2.45GHz EMR (1h/day for 5 days/week) from pregnancy to 3 weeks of age. Brain cortex and liver samples were taken from the newborns between the first and third weeks. In the EMR groups, lipid peroxidation levels in the brain and liver were increased following EMR exposure; however, the glutathione peroxidase (GSH-Px) activity, and vitamin A, vitamin E and β-carotene concentrations were decreased in the brain and liver. Glutathione (GSH) and vitamin C concentrations in the brain were also lower in the EMR groups than in the controls; however, their concentrations did not change in the liver. In conclusion, Wi-Fi-induced oxidative stress in the brain and liver of developing rats was the result of reduced GSH-Px, GSH and antioxidant vitamin concentrations. Moreover, the brain seemed to be more sensitive to oxidative injury compared to the liver in the development of newborns.

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KEYWORDS: Antioxidant vitamins; Brain; Electromagnetic radiation; Glutathione; Liver; Oxidative stress

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- Liver antioxidant stores protect the brain from electromagnetic field-induced oxidative stress in rats during pregnancy and the development of offspring. [J Matern Fetal Neonatal Med. 2...]
- Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats. [Indian J Ophthalmol. 2014]
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- Abstract

Appl Biochem Biotechnol. 2012 Jan;166(2):379-88. doi: 10.1007/s12010-011-9433-6. Epub 2011 Nov 29.

Pathophysiology of microwave radiation: effect on rat brain.

Kesari KK[1], Kumar S, Behari J.

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Abstract

The study aims to investigate the effect of 2.45 GHz microwave radiation on Wistar rats. Rats of 35 days old with 130 ± 10 g body weight were selected for this study. Animals were divided into two groups: sham exposed and experimental (six animals each). Animals were exposed for 2 h a day for 45 days at 2.45 GHz frequency (power density, 0.21 mW/cm(2)). The whole body specific absorption rate was estimated to be 0.14 W/kg. Exposure took place in a ventilated plexiglas cage and kept in an anechoic chamber under a horn antenna. After completion of the exposure period, rats were killed, and pineal gland and whole brain tissues were isolated for the estimation of melatonin, creatine kinase, caspase 3, and calcium ion concentration. Experiments were performed in a blind manner and repeated. A significant decrease ($P < 0.05$) was recorded in the level of pineal melatonin of exposed group as compared with sham exposed. A significant increase ($P < 0.05$) in creatine kinase, caspase 3, and calcium ion concentration was observed in whole brain of exposed group of animals as compared to sham exposed. One-way analysis of variance method was adopted for statistical analysis. The study concludes that a reduction in melatonin or an increase in caspase-3, creatine kinase, and calcium ion may cause significant damage in brain due to chronic exposure of these radiations. These biomarkers clearly indicate possible health implications of such exposures.

- Abstract

Biol Trace Elem Res. 2015 Feb;163(1-2):193-201. doi: 10.1007/s12011-014-0175-5. Epub 2014 Nov 14.

Effects of prenatal and postnatal exposure of Wi-Fi on development of teeth and changes in teeth element concentration in rats. [corrected].

Çiftçi ZZ[1], Kırzıoğlu Z, Nazıroğlu M, Özmen Ö.

Author information

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Erratum in

- Erratum to: Effects of prenatal and postnatal exposure of Wi-Fi on development of teeth and changes in teeth element concentration in rats. [Biol Trace Elem Res. 2015]

Abstract

The present study determined the effects of prenatal and postnatal exposure to Wi-Fi (2.45 GHz)-induced electromagnetic radiation (EMR) on tooth and surrounding tissue development as well as the element levels in growing rats. Twenty-four rats and their offspring were equally divided into two separate groups identified as experiment and control. The experiment group was exposed to 2.45 GHz EMR for 2 h/day during the periods of pregnancy (21 days) and lactation (21 days). The offspring of these dams were also exposed to EMR up to decapitation. The control group was exposed to cage stress for 2 h per day using the same protocol established for the experimental group. On the 7th, 14th, and 21st days after birth, 8 male offspring rats from each of the two groups were decapitated, and the jaws were taken for histological and immunohistochemical examination. Caspase-3 (1/50 dilution) was used in the immunohistochemical examination for apoptotic activity. On the last day of the experiment, the rats' incisors were also collected. In samples that were histologically and immunohistochemically examined, there was an increase in apoptosis and caspase-3 in both the control and the Wi-Fi groups during the development of the teeth. However, no significant difference was observed between the two groups in terms of development and apoptotic activity. Results from the elemental analysis showed that iron and strontium concentrations were increased in the Wi-Fi group, whereas boron, copper, and zinc concentrations were decreased. There were no statistically significant differences in calcium, cadmium, potassium, magnesium, sodium, or phosphorus values between the groups. Histological and immunohistochemical examinations between the experimental and control groups showed that exposure to 2.45 GHz EMR for 2 h per day does not interfere with the development of teeth and surrounding tissues. However, there were alterations in the elemental composition of the teeth, especially affecting such oxidative stress-related elements as copper, zinc, and iron, suggesting that short-term exposure to Wi-Fi-induced EMR may cause an imbalance in the oxidative stress condition in the teeth of growing rats.

Andrologia. 2014 Feb;46(1):65-72. doi: 10.1111/and.12044. Epub 2012 Nov 12.

Protective effects of melatonin against oxidative injury in rat testis induced by wireless (2.45 GHz) devices.

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Abstract

Wireless devices have become part of everyday life and mostly located near reproductive organs while they are in use. The present study was designed to determine the possible protective effects of melatonin on oxidative stress-dependent testis injury induced by 2.45-GHz electromagnetic radiation (EMR). Thirty-two rats were equally divided into four different groups, namely cage control (A1), sham control (A2), 2.45-GHz EMR (B) and 2.45-GHz EMR+melatonin (C). Group B and C were exposed to 2.45-GHz EMR during 60 min day(-1) for 30 days. Lipid peroxidation levels were higher in Group B than in Group A1 and A2. Melatonin treatment prevented the increase in the lipid peroxidation induced by EMR. Also reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) levels in Group D were higher than that of exposure group. Vitamin A and E concentrations decreased in exposure group, and melatonin prevented the decrease in vitamin E levels. In conclusion, wireless (2.45 GHz) EMR caused oxidative damage in testis by increasing the levels of lipid peroxidation and decreasing in vitamin A and E levels. Melatonin supplementation prevented oxidative damage induced by EMR and also supported the antioxidant redox system in the testis.

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Abstract

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Arch Dermatol Res. 2012 Sep;304(7):521-7. doi: 10.1007/s00403-012-1205-9012-1205-9. Epub 2012 Jan 12.

Protective effects of β-glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats.

Ceyhan AM[1], Akkaya VB, Güleçol •C, Ceyhan BM, Özgüner F, Chen W.

Author information

Abstract

In recent times, there is widespread use of 2.45-GHz irradiation-emitting devices in industrial, medical, military and domestic application. The aim of the present study was to investigate the effect of 2.45-GHz electromagnetic radiation (EMR) on the oxidant and antioxidant status of skin and to examine the possible protective effects of β-glucans against the oxidative injury. Thirty-two male Wistar albino rats were randomly divided into four equal groups: control; sham exposed; EMR; and EMR + β-glucan. A 2.45-GHz EMR emitted device from the experimental exposure was applied to the EMR group and EMR + β-glucan group for 60 min daily, respectively, for 4 weeks. β-glucan was administered via gavage at a dose of 50 mg/kg/day before each exposure to radiation in the treatment group. The activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the concentration of malondialdehyde (MDA) were measured in tissue homogenates of the skin. Exposure to 2.45-GHz EMR caused a significant increase in MDA levels and CAT activity, while the activities of SOD and GSH-Px decreased in skin tissues. Systemic β-glucan significantly reversed the elevation of MDA levels and the reduction of SOD activities. β-glucan treatment also slightly enhanced the activity of CAT and prevented the depletion of GSH-Px activity caused by EMR, but not statistically significantly. The present study demonstrated the role of oxidative mechanisms in EMR-induced skin tissue damages and that β-glucan could ameliorate oxidative skin injury via its antioxidant properties.

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Fertil Steril. 2012 Jan;97(1):39-45.e2. doi: 10.1016/j.fertnstert.2011.10.012. Epub 2011 Nov 23.

Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation.

Avendaño C[1], Mata A, Sanchez Sarmiento CA, Doncel GF.

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Abstract

OBJECTIVE:

To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa.

DESIGN:

Prospective in vitro study.

SETTING:

Center for reproductive medicine.

PATIENT(S):

Semen samples from 29 healthy donors.

INTERVENTION(S):

Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop.

MAIN OUTCOME MEASURE(S):

Evaluation of sperm motility, viability, and DNA fragmentation.

RESULT(S):

Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups.

CONCLUSION(S):

To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

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Comment in

- Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. [Fertil Steril. 2012]
- Laptop computers with Wi-Fi decrease human sperm motility and increase sperm DNA fragmentation. [Fertil Steril. 2012]
- Words of wisdom. Re: Use of laptop computers connected to Internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. [Eur Urol. 2012]

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Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants

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Abstract

Influence of environmental stress factors on both crop and wild plants of nutritional value is an important research topic. The past research has focused on rising temperatures, drought, soil salinity and toxicity, but the potential effects of increased environmental contamination by human-generated electromagnetic radiation on plants have little been studied. Here we studied the influence of microwave irradiation at bands corresponding to wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, essential oil content and volatile emissions in *Petroselinum crispum*, *Apium graveolens* and *Anethum graveolens*. Microwave irradiation resulted in thinner cell walls, smaller chloroplasts and mitochondria, and enhanced emissions of volatile compounds, in particular, monoterpenes and green leaf volatiles. These effects were stronger for WLAN-frequency microwaves. Essential oil content was enhanced by GSM-frequency microwaves, but the effect of WLAN-frequency microwaves was inhibitory. There was a direct relationship between microwave-induced structural and chemical modifications of the three plant species studied. These data collectively demonstrate that human-generated microwave pollution can potentially constitute a stress to the plants.

Keywords

Microwave; abiotic stress; essential oils; volatile organic compounds; aromatic plants

INTRODUCTION

Aromatic plants represent an important resource for human nutrition, due to their valuable properties, including medicinal benefits (Bonjar, 2004; Wong and Kitts, 2006; Bakkali et al., 2008; Ortan et al., 2009; Cornara et al., 2009). Therefore, understanding their chemical composition and how the properties of aromatic plants are affected by key climate change factors as well as human-generated pollution are research topics of major interest.

The key property of aromatic plants is the presence of essential oils that play important roles in plants acting as direct defenses against pathogen and herbivore attacks (Rhoades, 1977;

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Lewinsohn, 1991; Fugmann et al., 1997; Reddy et al., 2001). The essential oils are very complex natural mixtures that consist of molecules produced through different secondary metabolic pathways, characteristically containing terpenoids, benzenoids and sometimes aliphatic compounds (Bauer et al., 1998; Eggersdorfer, 1998; Cheng et al., 2007; Bakkali et al., 2008).

Both the composition and content of essential oils has been shown to strongly depend on plant species and environmental conditions (Langlille and MacLean, 1976; Letchamo and Gosselin, 1996; Zabaras et al., 2002; Manzan et al., 2003). These aspects are relevant because plants in natural conditions as well as in agricultural fields are exposed to a plethora of abiotic and biotic stresses and the importance of several biological and environmental stresses is expected to increase in the future (Peñuelas and Estiarte, 1998; Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009; Niinemets, 2010b).

The key abiotic stresses(Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009) of contemporary economical importance for plant growth worldwide are drought, heat, cold (chilling and freezing), high salinity, soil mineral deficiency and toxicity. Furthermore, diffuse environmental pollution, including air and soil pollution constitutes a major problem for agriculture and human health (Gauderman et al., 2004; WHO, 2004; Copaciu et al., 2013; Opriş et al., 2013). It was demonstrated that the blend of volatile organic compounds emitted by aromatic plants under stress factors is complex (Rodrigues-Navas et al., 2012). The complexity of volatile emissions in species having specialized storage structures for volatiles results from the circumstance that there may be emissions directly coming from storage and de novo emissions independent of storage (Staudt et al., 1997; Niinemets et al., 2010b; Monson et al., 2012; Grote et al., 2013; Li and Sharkey, 2013).

Among the novel potential pollution sources is the enhanced use of mobile phones and wireless devices generating an exponentially increased level of electromagnetic radiation in the microwave range of radiation frequencies (1-100 GHz). There have been some studies on microwave effects on plants showing no significant effects, while others have demonstrated important modifications in plant functioning. Laboratory growth experiments in plants subject to magnetic fields demonstrated that plants were taller and heavier (Martínez et al., 2003). Likewise, germination of *Cicer arietinum* L. seeds and early development were enhanced upon exposure to a moderate magnetic field (Vashisth and Nagarajan, 2008). It has been shown that electromagnetic radiation at broadcast-frequency (0.2-30 MHz) altered the cellular contents of calcium and sulfur, effect associated with the power of radiation (Balmori Martínez, 2003), while in animal cells has been observed that microwaves (frequency of 147 MHz, amplitude-modulated at 16 Hz) can influence the intercellular communication through altering the functioning of the calcium channels (Balmori Martínez, 2003). Exposure to microwaves (frequency of 9.75 GHz and low intensity) of wheat (*Triticum aestivum*) plants has resulted in cytogenetic changes (Pavel et al., 1998; Balmori Martínez, 2003). Studies have also shown alterations in condensed chromatin distribution of meristem cells exposed to low magnetic fields (Belyavskaya, 2001; Belyavskaya, 2004). In general, these studies collectively suggest that the effects of electromagnetic fields on plants can be variable.

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It is, however, unclear what the mechanism of low-energy microwave irradiation effects on plant is. While high energy microwave-radiation can break the chemical bonds (Caldwell et al., 1995; Barnes and Cardoso-Vilhena, 1996), the quantum energy of microwave radiation is low and mainly can have thermal effects, heating up selectively plant structures and possibly also alter the conformation of biomolecules, such as proteins, nucleic acids and membrane lipids. Furthermore, modifications in biomolecular tertiary structure can importantly alter the rate of physiological processes, again implying that microwaves can lead to stress conditions in plants (Takeuchi and Thornber, 1994; Ha et al., 1997; Havaux, 1998). Thus, it is important to gain more conclusive insight into the effects of microwaves on plant performance.

The aim of the present study was to investigate the influence of microwave irradiation on the ultrastructure of leaves, the essential oil content and volatile organic compounds emission of three aromatic plant species of the Apiaceae family, parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L. subsp. *hortorum* Alef.) and celery (*Apium graveolens* L.). The stress application consisted in three weeks microwave irradiation of plants at bands corresponding to wireless router (WLAN) and mobile devices (GSM). As the emissions of stress volatiles such as green leaf volatiles (GLV) and specific terpenes are enhanced upon exposure to different stresses (Heiden et al., 2003; Beauchamp et al., 2005; Copolovici et al., 2011; Niinemets et al., 2013), we hypothesized that microwave irradiation leads to enhanced emission of stress volatiles. In addition, we intend to investigate how microwave irradiation affects the leaf structure, content and composition of essential oils in these aromatic plants of nutritional and medicinal importance.

MATERIALS AND METHODS

Plant material and growth conditions

Plant material including parsley (*Petroselinum crispum* cv. Plain leaved 2) (P), dill (*Anethum graveolens subsp. hortorum* cv. Common) (D) and celery (*Apium graveolens* cv. Pascal Giant) (C) were grown in laboratory from seeds obtained from Agrosel (Câmpia Turzii, Romania). Fifteen seeds were sown in 150 mL plastic pots (height x diameter of 8.5 × 6.5 cm) filled with commercial garden soil.

Three weeks after seeding, the vessels with plants were placed in three identical anechoic chambers (Surducan et al., 2012) characterized by a degree of isolation of 60 dB at radio-frequency range between the exterior and interior. The fully-closed chambers were maintained under the same conditions of light intensity at 300 μmol m^{-2} s^{-1} (provided from four 4W MR16 LED lamps (every lamp consisting of 26 warm white SMD 5050 LED at 3300K), temperature (25°C), CO_2 concentration (385 ± 20 ppmv) and humidity (65%). One chamber was for non-treated control plants, while plants in the other two chambers were subjected to microwave irradiation. The microwave irradiation was performed at bands corresponding to mobile devices (GSM) using a modified AP5200 generator (D-LINK, China), operating in four bands (860 – 910 MHz frequency range, Pout 29 dBm), and to wireless router (WLAN) using a D-LINK wireless router 802.11g/2.4 GHz (2.412 – 2.48 GHz frequency range, Pout 19 dBm). In the irradiation chamber there is one stick antenna placed in the center of the ceiling. The exposure levels where chosen in agreement with the

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microwave irradiation levels measured in open space for heavily used GSM networks (100mW/m^2) and for indoor WLAN (70mW/m^2) communication protocols. The power density to the base of chambers was measured with a spectrum analyzer SPECTRAN HF 4060, AARONIA AG (Germany). Both control and microwave-irradiated plants were watered every 2 days with 10 mL of bidistilled water (bidistillator AcquaMatic model AWC/4D, Hamilton Laboratory Glass Ltd., Kent, UK).

Irradiation was performed during three weeks, after which plants were removed from the chambers for measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content. All measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content have been replicated with eight different plants.

Transmission electron microscopy measurements

Samples for transmission electron microscopy (TEM) were contrasted with 2% uranyl acetate in 50% ethanol solution for 2 min and in 0.2% lead citrate in 0.1 M sodium hydroxide solution for 2 min. The samples were dehydrated in ethanol series and embedded in epoxy resin, Epon 812. The samples were cut in an Ultramicrotome, Leica UC6 with a diamond knife and the ultrathin samples (100 nm) were analyzed with a 120kV TEM Model JEM 1010 (Jeol USA Inc., Peabody, MA, USA). The number of chloroplasts, mitochondria, starch grains in the chloroplasts and nuclei were determined in the palisade mesophyll cells for replicate plants under each treatment.

Essential oil extraction

Samples of fresh plant material of 1 g were frozen in liquid nitrogen, pulverized and essential oils were extracted with 2 mL of 1:1 (v/v) mixture of HPLC-grade diethyl ether and *n*-hexane (Merck, Germany). For extraction, plant material was initially soaked for 10 minutes with the solvent mixture, and then extracted in an ultrasonic bath (Elmasonic S 15H, 37 kHz) for 30 minutes at 30°C. Each extraction was performed using five parallel samples. In all cases, extracts were decanted and filtered through nylon syringe filters (0.45 μm) before use.

Volatile organic compounds (VOC's) sampling and photosynthetic parameters determinations

VOC sampling was performed using a portable gas exchange system GFS-3000 (Waltz GmbH, Effeltrich, Germany). The system has an environment-controlled cuvette with 8 cm^2 window area and multiple leaves were enclosed in the cuvette to fill the whole cuvette window. A volume of 4 L of air exiting the cuvette was sampled in a multibed stainless steel cartridge (8.88 × 0.65 cm, Supelco, Bellefonte, PA, USA) filled with Carbopack adsorbents (C 20/40 mesh, C 40/60 mesh, and X 20/40 mesh). The chamber air was sampled at a flow rate of 200 mL min^{-1} for 20 min using a 1003-SKC constant flow sampling pump (SKC Inc., Houston, TX, USA) at room temperature. Background air samples were taken before and after the measurements using the same system without the leaves enclosed in the cuvette. Using the same system, CO_2 and H_2O concentrations have been measured. The

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rates of net assimilation (A) and stomatal conductance to water vapour (g_s) were calculated per unit enclosed plant leaf area according to von Caemmerer and Farquhar (1981).

Essential oils and volatile organic compounds analysis

For both essential oils and VOC analysis, a Shimadzu QP2010 Plus gas chromatograph coupled with quadrupole mass spectrometer (GC–MS) (Kyoto, Japan) was used. The conditions for essential oils analysis were as follows: injector temperature was 215°C, initial oven temperature at 40°C was held for 1 min; ramped at 5°C min^{-1} up to 200°C, held at this temperature for 1 min; ramped at 10°C min^{-1} up to 220°C and held for further 5 min. Helium (purity 99.9999 %, Elmer Messer Gaas AS, Tallinn, Estonia) was employed as carrier gas with a constant flow rate of 1 mL min^{-1}. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 30 – 400, the transfer line temperature was set at 240°C and ion-source temperature at 150°C.

For VOC analysis, an automated cartridge desorber Shimadzu TD20 (Kyoto, Japan) was used. The volatiles were analyzed according to the method described in detail in Copolovici *et al*., 2009.

The essential oils and volatile organic compounds were identified by comparing the mass spectra of individual compounds with the spectra of GC purity external standards (Sigma Aldrich, St. Louis, MO, USA) and with the spectra of NIST Library.

Statistical analysis and data handling—For transmission electron microscopy (TEM) analyses, five replicate measurements, and for essential oils and volatile organic compound three replicates with independent samples of plants were available and we report means of the replications ± SE at each treatment. The means were statistically compared with Student ANOVAs followed by post hoc Tukey's tests using ORIGIN 8 (OriginLab Corporation, Northampton, MA, USA). All statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ultrastructural analyses and photosynthetic parameters

Irradiation resulted in both qualitative and quantitative modifications in leaf anatomy. Palisade and spongy-parenchyma cells exhibit slightly wavy walls in leaves of irradiated plants, while in leaves of control plants were straight-walled (Fig. 1).

This indicates alterations in spatial arrangement of cells in leaf lamina cross-section and a moderate decrease of lamina turgidity. In fact, cell wall thickness was reduced by microwave-irradiation, and generally more strongly by the treatment with WLAN-frequency microwaves (Table 1).

Chloroplasts retained their ultrastructure and normal arrangement within cells, but chloroplasts tended to be smaller in irradiated leaves, especially in the case of WLAN frequency microwaves (Table 1). The ratio of starch grain area to chloroplast area was somewhat increased in treatment with GSM-frequency microwaves in *P. crispum* and *A. graveolens*, while it was reduced in WLAN-frequency microwave treatment in *P. crispum*

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(Table 1). Nevertheless, the differences among the treatments were relatively small, indicating moderate effects of microwave treatments on starch accumulation.

The mitochondrion length generally decreased in microwave-treated plants, especially in the case of plants irradiated with WLAN-frequency microwaves (Table 1). In addition, the number of mitochondrial cristae was also somewhat less, suggesting a certain decrease in their metabolic activity. Nuclei of most cells showed normal structure, however abundance of heterochromatin and presence of wavy contours tended to be greater in microwave-treated plants.

The anatomical modifications were qualitatively similar among species (Table 1). In all species, the treatment with WLAN-frequency microwaves resulted in greater anatomical changes than the treatment with GSM-frequency microwaves (Table 1). However, the microwave-induced changes were the strongest in *Anethum graveolens*, where all treatments differed from each other (Table 1) followed by *P. crispum* and *Apium graveolens*. In this species, chloroplast length did not differ among the treatments and mitochondrion length in GSM-frequency microwaves was not different from that in control plants (Table 1).

These data collectively demonstrate important alterations in foliage ultrastructure by microwave irradiation, and are in agreement with observations in wheat (*Triticum aestivum*) exhibiting pronounced cytogenetic changes in response to microwaves (Pavel et al., 1998; Balmori Martínez, 2003). It was shown that under the influence of low-intensity microwaves in the species of *Triticum aestivum*, as compared to the control plants different types of chromosomal aberrations appeared: delayed chromosomes, micronuclei, interchromosomal bridges, chromosomal fragments (Pavel et al., 1998). In meristem cells of *Pisum sativum* L. roots exposed to low-magnetic field were observed ultrastructural changes such as a noticeable accumulation of lipid bodies, development of a lytic compartment (vacuoles, cytosegresomes and paramural bodies), and reduction of phytoferritin in plastids. The most sensitive organelle to low-magnetic field application was mitochondria, whose size and relative volume in cells increased, matrix was electron-transparent, and cristae reduced (Belyavskaya, 2001).

Photosynthesis parameters (assimilation rates and stomata conductance to water vapor) have been affected by microwaves exposure (Figure 2). Even more, both parameters are influenced by the strength of the stress.

Overall, reduction in the size of organelles may indicate that photosynthesis and respiratory metabolism was somewhat impaired by microwave treatment (Louwerse and van der Zweerde, 1977; Lichtenthaler, 1981; Griffin et al., 2001; Terashima et al., 2011). On the other hand, reduction in cell wall thickness reduces mesophyll diffusion conductance to chloroplasts thereby potentially compensating for reduced physiological potentials and altered stomatal conductance to water vapor (Terashima et al., 2006; Tosens et al., 2012a; Tosens et al., 2012b; Tomás et al., 2013). However, reduced cell wall thickness reduces cellular resistance to low leaf water potentials (Niinemets, 2001). Thus, microwave irradiance may importantly decrease plant drought resistance.

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Changes in essential oils content in response to microwave irradiation: general patterns

Many aromatic plants have specialized structures for the storage of volatiles and the composition of essential oils in these storage structures is often complex (Letchamo et al., 1995; Manzan et al. 2003; Rajabi et al., 2013). Our study also observed complex composition of essential oils in the studied species, 10 compounds were detected in *P. crispum*, 11 compounds in *Anethum graveolens* and 7 compounds in *Apium graveolens*. In all species, monoterpenes constituted a significant component of the essential oil (Fig. 3). In addition, several specific benzenoids were also dominating components of the oil: apiol in *P. crispum*, and myristicin and dillapiole in *Anethum graveolens* (Fig. 3). Lipoxygenase pathway compounds were important constituents in essential oil in *P. crispum* and *Apium graveolens* (Fig. 3). In addition, the main volatile compounds in *Apium* graveolens are 3-hexen-1-ol, myrcene, α-ocimene, γ-terpinene (Fig. 3). These results broadly agree with past observations of essential oils produced by these species (Deng et al., 2003; Orav et al., 2003).

Multiple environmental factors have been shown to modify the content of essential oil (Langlille and MacLean, 1976; Gershenzon, 1984; Letchamo et al., 1994; Wannaz et al., 2003; Peñuelas et al., 2011). Such modifications in essential oil content have been often explained on the basis of hypotheses linking growth, and primary and secondary metabolism (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). According to these hypotheses, when sink activity (growth) rate decreases and carbon dioxide availability is in excess of that naturally occurring in the atmosphere, plants increase the rate of synthesis of secondary metabolites such as essential oils (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). In agreement with this hypothesis, water deficit has been shown to increase the yield of essential oil and affected its relative composition in *P. crispum* (Petropoulos, 2008). Analogously, enhanced salinity increased the essential oil yield for *Anethum graveolens* plants under stress (Ghassemi-Golezani et al., 2011).

In our study, microwave irradiation by GSM-frequency microwaves generally increased the essential oil contents (Fig. 3), while the effect of WLAN-frequency microwaves was less clear, varying from positive or negative for different compounds and species (Fig. 3). In a like manner, ozone stress induced two distinct pathways in *P. crispum* (Eckey-Kaltenbach et al., 1994) suggesting that different types and severity of stress can lead to qualitatively different responses. Taken together, these results are in partial agreement with several past observations indicating enhanced production of essential oils under stress and also are in agreement with the evidence of impaired photosynthetic and respiratory metabolism under microwave irradiation (Table 1). However, differently from essential oils, the anatomical modifications were significant under WLAN microwave irradiation (cf. Table 1 and Fig. 1c).

Species and microwave-frequency effects on essential oils

Although the effects were broadly similar among species, important species differences were observed in individual compound responses to GSM- and WLAN-frequency microwaves. Among the three plant species tested in these experiments, the strongest effects

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of microwave irradiation on essential oils were observed on *Anethum graveolens* plants (Fig. 3).

For individual compounds in *P. crispum*, the microwave irradiation produced by GSM generator statistically increased 3-hexen-1-ol, myrcene, α-phellandrene, β-phellandrene, myristicin and apiole contents. Compared to the reference, the strongest increase in response to GSM-frequency irradiation was observed for apiole (more than seven times greater content, Fig. 3a). The WLAN-frequency microwaves statistically increased the content of α-pinene, β-phellandrene, myristicin and apiol in this species (Fig. 3a).

In *Anethum graveolens* irradiated with GSM microwaves, increased content was observed for β-pinene, α-phellandrene and dillapiole (Fig. 3b). However, WLAN-frequency microwaves reduced α-phellandrene, myristicin and dillapiole content, whereas the greatest reduction was observed for myristicin (approximately to the level 18% of that in reference plants, Fig. 3b).

In *Apium graveolens*, both types of microwaves used in this study increased 3-hexen-1-ol content (Fig. 3c). Irradiation by WLAN-frequency microwaves reduced myrcene (19%) and α-ocimene (21%) contents (Fig. 3c).

Species-differences in environmental responses to stress factors have been demonstrated (El-Keltawi and Croteau, 1986; Mangas et al., 2006) although interspecific studies have been rare. Species ranking according to anatomical modifications was similar to the ranking based on essential oil changes (cf. Table 1 and Fig. 3). The structure of *Apium graveolens* leaves was the least affected by microwave irradiation and the effect on leaf chemistry was also the least in this species.

General patterns in volatile organic compounds emissions

Our data demonstrate that the emissions observed did reflect a mixture of both storage emission consisting of compounds present in essential oils and de novo emissions. The blend of volatiles was very complex and, in all plant species, the non-stressed plants also emitted monoterpenes and benzenoids present in essential oils, in some cases even compounds not-present in essential oils (Fig. 4). The number of compounds detected in the emissions was greater than in the essential oils, and characteristic de novo released stress volatiles were observed (Fig. 4). 16 compounds were detected in the emissions of *P. crispum*, 16 compounds in *Anethum graveolens* and 20 compounds in *Apium graveolens.*

There was evidence of similar enhancement of essential oils and emissions for several monoterpenes, especially for GSM microwave treatments in *P. crispum* and *Anethum graveolens* (cf. Figs. 3 and 4). However, in these species, emissions were more strongly enhanced under WLAN microwave treatment, which appeared to have an inhibitory effect on the content of the same terpenoids, e.g. α-pinene and β-phellandrene (cf. Figs. 3 and 4). Although there was evidence of parallel changes in contents and emissions for some volatiles in species, and for some treatments, this evidence suggests that the storage and de novo emissions cannot be fully teased apart in the current study. Nevertheless, the data

suggest that the total emissions and especially treatment differences reflect to a large degree the microwave-induced de novo synthesized plant volatiles.

Compound-class, species- and treatment-specific differences in volatile emissions

Among the de novo emissions, green leaf volatiles (GLV), also called volatiles of lipoxygenase pathway (LOX volatiles) are released in plants in response to different stresses (Copolovici and Niinemets, 2010; Copolovici et al., 2011; Copolovici et al., 2012). GLVs are formed in the hydroperoxide lyase pathway of oxylipin metabolism from free octadecanoic fatty acids and consist usually of a mixture of C6 aldehydes and ketones (Matsui, 2006). In our study, all microwave-irradiated plants emitted the following GLVs: (*E*)-2-hexenal, (*Z*)-3-hexenol, 1-hexanol, while the emissions of GLVs were very low at the level of detection limit of our device in control plants (Fig. 4).

In general, in all plant species studied, the emissions of GLV were greater for WLAN-frequency microwaves compared to GSM-frequency microwaves (Fig. 4, $P < 0.001$ for all). These results suggest greater stress in the case of WLAN microwave irradiation, and are in agreement with the more significant changes in anatomy of leaves induced by WLAN microwaves (Table 1). Stronger GLV emissions under more severe stress have been shown for water (Capitani et al., 2009), ozone (Beauchamp et al., 2005), herbivory attack (Allmann and Baldwin, 2010) and temperature (Copolovici et al., 2012) stresses.

The GLV emissions of the *P. crispum* and *Anethum graveolens* were dominated by the 1-hexanol (Fig. 4), while in *Apium graveolens* the main component was (*Z*)-3-hexenol that was also important constituent in the essential oil in this species (Figs. 3 and 4). The total GLV emission from *P. crispum* and *Anethum graveolens* was five times higher than from *Apium graveolens*. As with the essential oil content (Fig. 3), *Apium graveolens* was clearly less sensitive to the microwave fields than *P. crispum* and *Anethum graveolens*.

The monoterpenes detected in the emissions were α-pinene, β-pinene, camphene, limonene, 3-carene, *para*-cymene, β-phellandrene, (*E*)-β-ocimene, eucalyptol and bornyl acetate. In *P. crispum*, emission of α-pinene, β-pinene and β-phellandrene were dominant and enhanced by microwave irradiation, especially in the case of WLAN-frequency microwave treatment (Fig. 4a). Treatment effects on monoterpene emissions were similar for *Apium graveolens* and *Anethum graveolens*, but the main components are at some extent different (Figure 4b and 4c). Monoterpene emissions from *Anethum graveolens* were dominated by α-pinene, α-phellandrene and limonene, and these emissions were enhanced by microwave irradiation (Fig. 4b). In *Apium graveolens*, the emissions were almost four times lower than in the other species and were dominated by α-pinene, β-pinene and limonene (Fig. 4c). The emission of terpenes was inhibited by microwave irradiation similarly to the content of essential oils (Figs. 3 and 4).

Overall, these emitted monoterpenes are characteristic plant-released compounds and are not specific to stress-induced emissions (Staudt et al., 1997; Kesselmeier and Staudt, 1999; Staudt et al., 2000; Niinemets et al., 2010b). However, the emission rates of these typical monoterpenes is also often enhanced in stress conditions (Vuorinen et al., 2004; Blande et al., 2007; Heijari et al., 2008; Copolovici et al., 2011; Copolovici et al., 2012), implying that

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induced and constitutive emission are often difficult to separate. Among the characteristic induced monoterpenes (Staudt and Bertin, 1998; Hakola et al., 2001; Noe et al., 2006; Niinemets et al., 2010b), emissions of (*E*)-β-ocimene and 1,8-cineole were strongly enhanced by microwave-irradiation in *Anethum graveolens* (Fig. 4). In addition, both *P. crispum* and *Anethum graveolens*, emitted in low amounts longicyclene, a stress induced sesquiterpene, under WLAN-frequency irradiation.

CONCLUSIONS

The presented data collectively suggest that microwave irradiation constitute a stress to the plants, resulting in enhanced emissions of green leaf volatiles, up-regulation of terpenoid emissions and modification in essential oil content and foliage anatomy. Anatomical and emission traits suggested that WLAN-frequency irradiation resulted in more severe stress than GSM-frequency irradiation, but the effect of WLAN-frequency irradiation on essential oil was inhibitory. There was an agreement between anatomical and chemical traits with anatomically most resistant species *Apium graveolens* being chemically least responsive.

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Abbreviations

VOC	volatile organic compounds
GLV	green leaf volatiles
WLAN	wireless router
GSM	mobile devices
TEM	transmission electron microscopy

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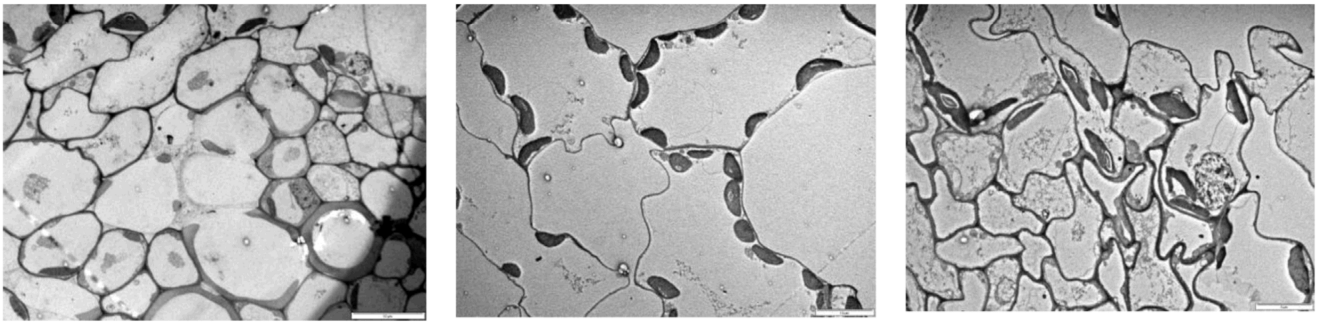

Fig. 1.

TEM images of cell walls in leaves of microwave-irradiated and control parsley: a) Control; b) GSM irradiated; c) WLAN iradiated.

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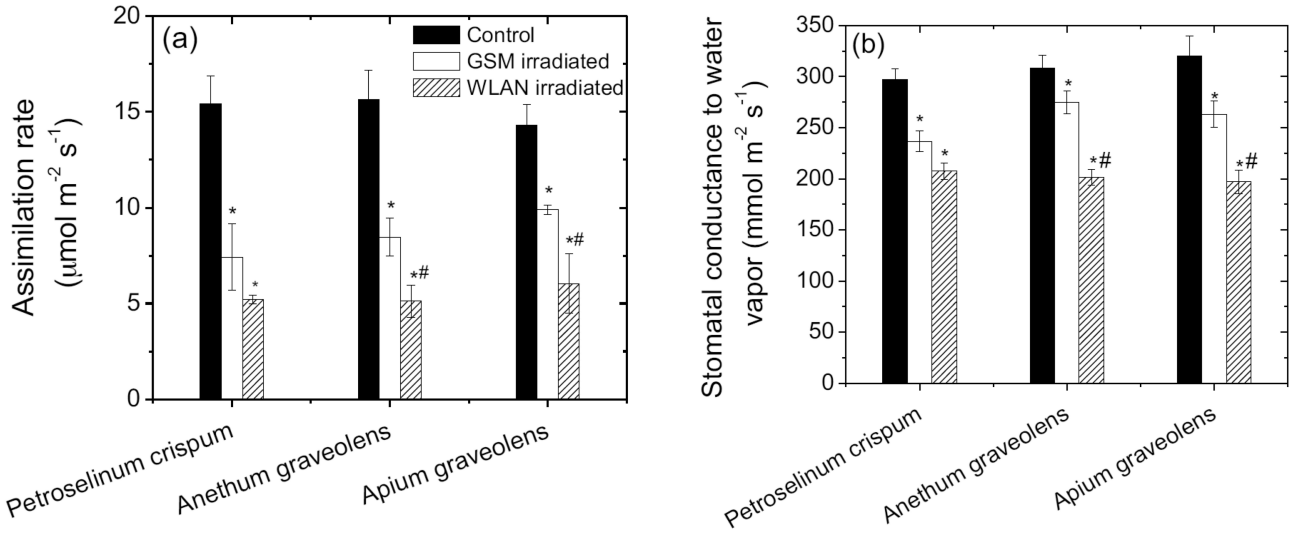

Fig. 2.

Changes in net assimilation rate (a) and stomatal conductance to water vapour (b) in 3 aromatic plants in response to microwave stress. The data are expressed per unit projected leaf area. Each data point is the mean (± SE) of 8 independent replicate experiments with a different plant.

* and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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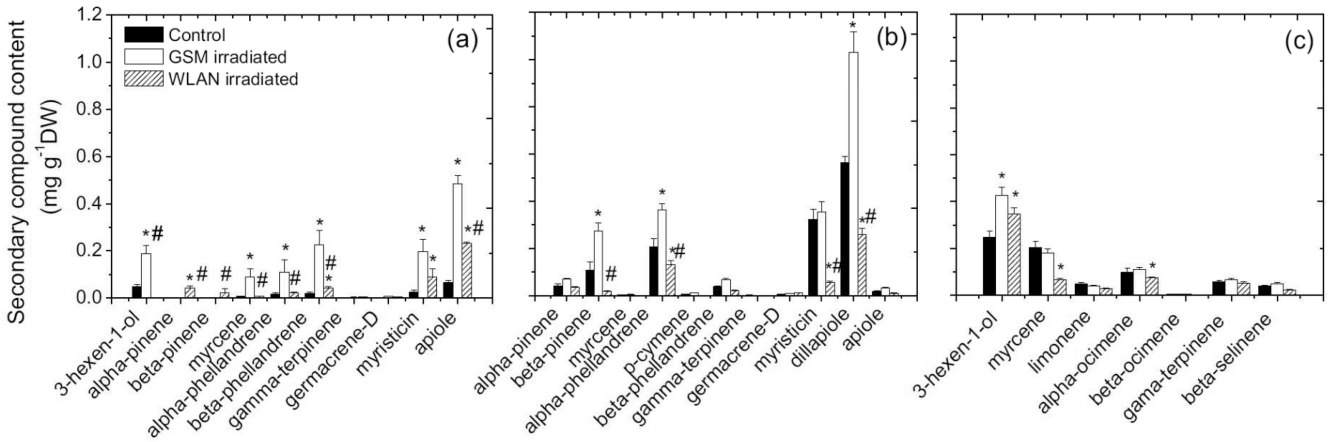

Fig. 3.

Changes in terpene content (mg g^{-1} FW) in *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) foliage in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM). Each data point is the mean (± SE) of three independent replicate experiments with a different plant. * and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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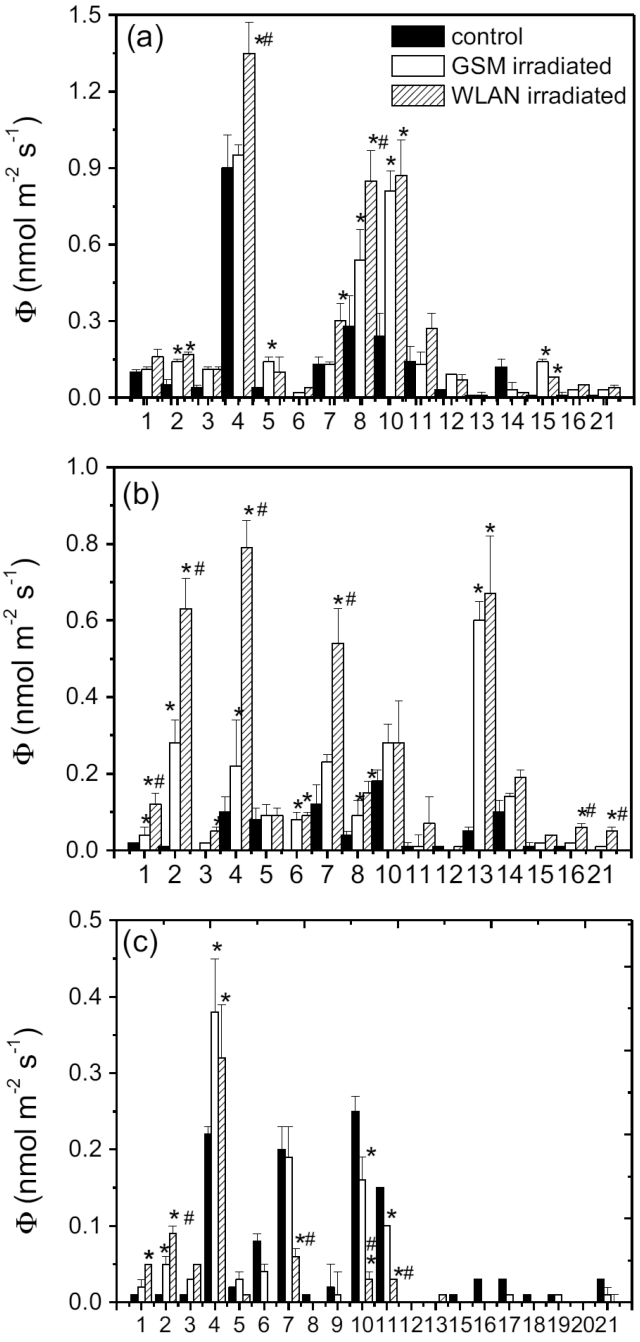

Fig. 4.

Alteration of the emission of volatile organic compounds (nmol m^{-2} s^{-1}) from foliage of *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM) (presentation of statistical differences as shown in Fig. 2). Each number corresponds to a particular volatile compound as follows: **1.** 1-hexanol; **2.** *(Z)*-3-hexen-1-ol; **3.** (*E*)-2-hexenal; **4.** α-pinene; **5.** camphene; **6.** β-myrcene; **7.** β-pinene; **8.** α-phellandrene; **9.** Δ-3-carene; **10.** D-limonene; **11.** *para*-cymene; **12.** β-phellandrene; **13.** (*E*)-

β-ocimene; **14.** 1,8-cineol; **15.** iso-bornyl acetate; **16.** longicyclene; **17.** caryophyllene oxide; **18.** α-selinene; **19.** (*Z*)-β-farnesene; **20.** α-caryophyllene; **21.** geranylacetone.

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Table 1

Ultrastructural analysis of the leaves of studied plants. The average values (± SE) are replicates of six independent measurements with different plants.

Treatment	Cell wall thickness (μm)	Chloroplast length (μm)	Chloroplast area (μm^2)	Mitochondrion length (μm)	Ratio of starch grain area to chloroplast area (%)
Petroselinum crispum					
Control	0.300 ± 0.07	6.78 ± 0.12	13.31 ± 0.22	1.00 ± 0.27	8.93 ± 0.13
860 – 910 MHz	0.250 ± 0.06	6.76 ± 0.28	8.491 ± 0.06	0.90 ± 0.13	9.99 ± 0.12
2.4 - 2.5 GHz	0.200 ± 0.05	6.50 ± 0.16	7.807 ± 0.11	0.70 ± 0.05	6.01 ± 0.08
Anethum graveolens					
Control	0.187 ± 0.01	5.90 ± 0.13	8.43 ± 0.23	1.68 ± 0.13	5.21 ± 0.13
860 – 910 MHz	0.175 ± 0.01	5.20 ± 0.17	8.08 ± 0.29	1.00 ± 0.25	8.13 ± 0.08
2.4 - 2.5 GHz	0.175 ± 0.01	4.85 ± 0.31	7.04 ± 0.22	0.80 ± 0.10	0
Apium graveolens					
Control	0.160 ± 0.01	5.80 ± 0.20	7.68 ± 0.14	1.57 ± 0.08	5.34 ± 0.28
860 – 910 MHz	0.156 ± 0.01	4.33 ± 0.26	7.06 ± 0.35a	0.55 ± 0.13	0
2.4 - 2.5 GHz	0.136 ± 0.01	3.33 ± 0.26	6.68 ± 0.14	0.25 ± 0.13	0

Electromagn Biol Med. 2015 Mar;34(1):63-71. doi: 10.3109/15368378.2013.871619. Epub 2014 Jan 24.

The effects of long-term exposure to a 2450 MHz electromagnetic field on growth and pubertal development in female Wistar rats.

Sangun O[1], Dundar B, Darici H, Comlekci S, Doguc DK, Celik S.

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Abstract

The aim of this study was to investigate the effects of a 2450 MHz electromagnetic field (EMF) (wireless internet frequency) on the growth and development of female Wistar rats. The study was conducted on three groups of rats. The prenatal and postnatal groups were exposed to EMF 1 h/day beginning from intrauterine and postnatal periods, respectively. The third group was the sham-exposed group. Growth, nutrition and vaginal opening (VO) were regularly monitored. Serum and tissue specimens were collected at puberty. Histological examinations, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) measurements in ovary and brain tissues and also immunohistochemical staining of the hypothalamus were performed besides the determination of serum FSH, LH, E2 and IGF-1 values. Birth masses of the groups were similar ($p > 0.05$). Mass gain per day was significantly lower and the puberty was significantly later in the prenatal group. Brain and ovary TOS and OSI values in the prenatal group were significantly increased ($p < 0.05$) compared to the control group. Serum LH levels of the prenatal and postnatal groups were increased, although serum FSH, and E2 values did not differ among the groups ($p > 0.05$). Histological examinations of the specimens revealed no statistically significant difference between the groups ($p > 0.05$). Exposure to 2450 MHz EMF, particularly in the prenatal period, resulted in postnatal growth restriction and delayed puberty in female Wistar rats. Increased TOS and OSI values in the brain and ovary tissues can be interpreted as a sign of chronic stress induced by EMF. This is the first longitudinal study which investigates the effects of EMF induced by wireless internet on pubertal development beside growth.

KEYWORDS:

2450 MHz; growth; puberty; wireless

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Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats.

Meena R[1], Kumari K, Kumar J, Rajamani P, Verma HN, Kesari KK.

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Microwave (MW) radiation produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may adversely affects the reproductive pattern. Present study aimed to investigate the protective effects of melatonin (is well known antioxidant that protects DNA, lipids and proteins from free radical damage) against oxidative stress-mediated testicular impairment due to long-term exposure of MWs. For this, 70-day-old male Wistar rats were divided into four groups (n = 6/group): Sham exposed, Melatonin (Mel) treated (2 mg/kg), 2.45 GHz MWs exposed and MWs + Mel treated. Exposure took place in Plexiglas cages for 2 h a day for 45 days where, power density (0.21 mW/cm(2)) and specific absorption rate (SAR 0.14 W/Kg) were estimated. After the completion of exposure period, rats were sacrificed and various stress related parameters, that is LDH-X (lactate dehydrogenase isoenzyme) activity, xanthine oxidase (XO), ROS (reactive oxygen species), protein carbonyl content, DNA damage and MDA (malondialdehyde) were performed. Result shows that melatonin prevent oxidative damage biochemically by significant increase ($p < 0.001$) in the levels of testicular LDH-X, decreased ($p < 0.001$) levels of MDA and ROS in testis ($p < 0.01$). Meanwhile, it reversed the effects of MWs on XO, protein carbonyl content, sperm count, testosterone level and DNA fragmentation in testicular cells. These results concluded that the melatonin has strong antioxidative potential against MW induced oxidative stress mediated DNA damage in testicular cells.

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Yildirim ME[1], Kaynar M[2], Badem H[3], Cavis M[4], Karatas OF[4], Cimentepe E[4].

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Abstract

In this study, we aimed to assess the potential harmful effects of radiofrequency-electromagnetic radiation on sperm parameters. We requested semen for analyses from the male patients coming to our infertility division and also asked them to fill out an anonymous questionnaire. We queried their mobile phone and wireless Internet usage frequencies in order to determine their radiofrequency-electromagnetic radiation exposure. A total of 1082 patients filled the questionnaire but 51 of them were excluded from the study because of azoospermia. There was no significant difference between sperm counts and sperm morphology excluding sperm motility, due to mobile phone usage period, ($p = 0.074$, $p = 0.909$, and $p = 0.05$, respectively). The total motile sperm count and the progressive motile sperm count decreased due to the increase of internet usage ($p = 0.032$ and $p = 0.033$, respectively). In line with the total motile sperm count, progressive motile sperm count also decreased with wireless Internet usage compared with the wired Internet connection usage ($p = 0.009$ and $p = 0.018$, respectively). There was a negative correlation between wireless Internet usage duration and the total sperm count ($r = -0.089$, $p = 0.039$). We have also explored the negative effect of wireless Internet use on sperm motility according to our preliminary results.

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Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants

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Abstract

Influence of environmental stress factors on both crop and wild plants of nutritional value is an important research topic. The past research has focused on rising temperatures, drought, soil salinity and toxicity, but the potential effects of increased environmental contamination by human-generated electromagnetic radiation on plants have little been studied. Here we studied the influence of microwave irradiation at bands corresponding to wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, essential oil content and volatile emissions in *Petroselinum crispum*, *Apium graveolens* and *Anethum graveolens*. Microwave irradiation resulted in thinner cell walls, smaller chloroplasts and mitochondria, and enhanced emissions of volatile compounds, in particular, monoterpenes and green leaf volatiles. These effects were stronger for WLAN-frequency microwaves. Essential oil content was enhanced by GSM-frequency microwaves, but the effect of WLAN-frequency microwaves was inhibitory. There was a direct relationship between microwave-induced structural and chemical modifications of the three plant species studied. These data collectively demonstrate that human-generated microwave pollution can potentially constitute a stress to the plants.

Keywords

Microwave; abiotic stress; essential oils; volatile organic compounds; aromatic plants

INTRODUCTION

Aromatic plants represent an important resource for human nutrition, due to their valuable properties, including medicinal benefits (Bonjar, 2004; Wong and Kitts, 2006; Bakkali et al., 2008; Ortan et al., 2009; Cornara et al., 2009). Therefore, understanding their chemical composition and how the properties of aromatic plants are affected by key climate change factors as well as human-generated pollution are research topics of major interest.

The key property of aromatic plants is the presence of essential oils that play important roles in plants acting as direct defenses against pathogen and herbivore attacks (Rhoades, 1977;

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Lewinsohn, 1991; Fugmann et al., 1997; Reddy et al., 2001). The essential oils are very complex natural mixtures that consist of molecules produced through different secondary metabolic pathways, characteristically containing terpenoids, benzenoids and sometimes aliphatic compounds (Bauer et al., 1998; Eggersdorfer, 1998; Cheng et al., 2007; Bakkali et al., 2008).

Both the composition and content of essential oils has been shown to strongly depend on plant species and environmental conditions (Langlille and MacLean, 1976; Letchamo and Gosselin, 1996; Zabaras et al., 2002; Manzan et al., 2003). These aspects are relevant because plants in natural conditions as well as in agricultural fields are exposed to a plethora of abiotic and biotic stresses and the importance of several biological and environmental stresses is expected to increase in the future (Peñuelas and Estiarte, 1998; Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009; Niinemets, 2010b).

The key abiotic stresses(Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009) of contemporary economical importance for plant growth worldwide are drought, heat, cold (chilling and freezing), high salinity, soil mineral deficiency and toxicity. Furthermore, diffuse environmental pollution, including air and soil pollution constitutes a major problem for agriculture and human health (Gauderman et al., 2004; WHO, 2004; Copaciu et al., 2013; Opriş et al., 2013). It was demonstrated that the blend of volatile organic compounds emitted by aromatic plants under stress factors is complex (Rodrigues-Navas et al., 2012). The complexity of volatile emissions in species having specialized storage structures for volatiles results from the circumstance that there may be emissions directly coming from storage and de novo emissions independent of storage (Staudt et al., 1997; Niinemets et al., 2010b; Monson et al., 2012; Grote et al., 2013; Li and Sharkey, 2013).

Among the novel potential pollution sources is the enhanced use of mobile phones and wireless devices generating an exponentially increased level of electromagnetic radiation in the microwave range of radiation frequencies (1-100 GHz). There have been some studies on microwave effects on plants showing no significant effects, while others have demonstrated important modifications in plant functioning. Laboratory growth experiments in plants subject to magnetic fields demonstrated that plants were taller and heavier (Martínez et al., 2003). Likewise, germination of *Cicer arietinum* L. seeds and early development were enhanced upon exposure to a moderate magnetic field (Vashisth and Nagarajan, 2008). It has been shown that electromagnetic radiation at broadcast-frequency (0.2-30 MHz) altered the cellular contents of calcium and sulfur, effect associated with the power of radiation (Balmori Martínez, 2003), while in animal cells has been observed that microwaves (frequency of 147 MHz, amplitude-modulated at 16 Hz) can influence the intercellular communication through altering the functioning of the calcium channels (Balmori Martínez, 2003). Exposure to microwaves (frequency of 9.75 GHz and low intensity) of wheat (*Triticum aestivum*) plants has resulted in cytogenetic changes (Pavel et al., 1998; Balmori Martínez, 2003). Studies have also shown alterations in condensed chromatin distribution of meristem cells exposed to low magnetic fields (Belyavskaya, 2001; Belyavskaya, 2004). In general, these studies collectively suggest that the effects of electromagnetic fields on plants can be variable.

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It is, however, unclear what the mechanism of low-energy microwave irradiation effects on plant is. While high energy microwave-radiation can break the chemical bonds (Caldwell et al., 1995; Barnes and Cardoso-Vilhena, 1996), the quantum energy of microwave radiation is low and mainly can have thermal effects, heating up selectively plant structures and possibly also alter the conformation of biomolecules, such as proteins, nucleic acids and membrane lipids. Furthermore, modifications in biomolecular tertiary structure can importantly alter the rate of physiological processes, again implying that microwaves can lead to stress conditions in plants (Takeuchi and Thornber, 1994; Ha et al., 1997; Havaux, 1998). Thus, it is important to gain more conclusive insight into the effects of microwaves on plant performance.

The aim of the present study was to investigate the influence of microwave irradiation on the ultrastructure of leaves, the essential oil content and volatile organic compounds emission of three aromatic plant species of the Apiaceae family, parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L. subsp. *hortorum* Alef.) and celery (*Apium graveolens* L.). The stress application consisted in three weeks microwave irradiation of plants at bands corresponding to wireless router (WLAN) and mobile devices (GSM). As the emissions of stress volatiles such as green leaf volatiles (GLV) and specific terpenes are enhanced upon exposure to different stresses (Heiden et al., 2003; Beauchamp et al., 2005; Copolovici et al., 2011; Niinemets et al., 2013), we hypothesized that microwave irradiation leads to enhanced emission of stress volatiles. In addition, we intend to investigate how microwave irradiation affects the leaf structure, content and composition of essential oils in these aromatic plants of nutritional and medicinal importance.

MATERIALS AND METHODS

Plant material and growth conditions

Plant material including parsley (*Petroselinum crispum* cv. Plain leaved 2) (P), dill (*Anethum graveolens subsp. hortorum* cv. Common) (D) and celery (*Apium graveolens* cv. Pascal Giant) (C) were grown in laboratory from seeds obtained from Agrosel (Câmpia Turzii, Romania). Fifteen seeds were sown in 150 mL plastic pots (height x diameter of 8.5 × 6.5 cm) filled with commercial garden soil.

Three weeks after seeding, the vessels with plants were placed in three identical anechoic chambers (Surducan et al., 2012) characterized by a degree of isolation of 60 dB at radio-frequency range between the exterior and interior. The fully-closed chambers were maintained under the same conditions of light intensity at 300 μmol m^{-2} s^{-1} (provided from four 4W MR16 LED lamps (every lamp consisting of 26 warm white SMD 5050 LED at 3300K), temperature (25°C), CO_2 concentration (385 ± 20 ppmv) and humidity (65%). One chamber was for non-treated control plants, while plants in the other two chambers were subjected to microwave irradiation. The microwave irradiation was performed at bands corresponding to mobile devices (GSM) using a modified AP5200 generator (D-LINK, China), operating in four bands (860 – 910 MHz frequency range, Pout 29 dBm), and to wireless router (WLAN) using a D-LINK wireless router 802.11g/2.4 GHz (2.412 – 2.48 GHz frequency range, Pout 19 dBm). In the irradiation chamber there is one stick antenna placed in the center of the ceiling. The exposure levels where chosen in agreement with the

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microwave irradiation levels measured in open space for heavily used GSM networks (100mW/m^2) and for indoor WLAN (70mW/m^2) communication protocols. The power density to the base of chambers was measured with a spectrum analyzer SPECTRAN HF 4060, AARONIA AG (Germany). Both control and microwave-irradiated plants were watered every 2 days with 10 mL of bidistilled water (bidistillator AcquaMatic model AWC/4D, Hamilton Laboratory Glass Ltd., Kent, UK).

Irradiation was performed during three weeks, after which plants were removed from the chambers for measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content. All measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content have been replicated with eight different plants.

Transmission electron microscopy measurements

Samples for transmission electron microscopy (TEM) were contrasted with 2% uranyl acetate in 50% ethanol solution for 2 min and in 0.2% lead citrate in 0.1 M sodium hydroxide solution for 2 min. The samples were dehydrated in ethanol series and embedded in epoxy resin, Epon 812. The samples were cut in an Ultramicrotome, Leica UC6 with a diamond knife and the ultrathin samples (100 nm) were analyzed with a 120kV TEM Model JEM 1010 (Jeol USA Inc., Peabody, MA, USA). The number of chloroplasts, mitochondria, starch grains in the chloroplasts and nuclei were determined in the palisade mesophyll cells for replicate plants under each treatment.

Essential oil extraction

Samples of fresh plant material of 1 g were frozen in liquid nitrogen, pulverized and essential oils were extracted with 2 mL of 1:1 (v/v) mixture of HPLC-grade diethyl ether and *n*-hexane (Merck, Germany). For extraction, plant material was initially soaked for 10 minutes with the solvent mixture, and then extracted in an ultrasonic bath (Elmasonic S 15H, 37 kHz) for 30 minutes at 30°C. Each extraction was performed using five parallel samples. In all cases, extracts were decanted and filtered through nylon syringe filters (0.45 μm) before use.

Volatile organic compounds (VOC's) sampling and photosynthetic parameters determinations

VOC sampling was performed using a portable gas exchange system GFS-3000 (Waltz GmbH, Effeltrich, Germany). The system has an environment-controlled cuvette with 8 cm^2 window area and multiple leaves were enclosed in the cuvette to fill the whole cuvette window. A volume of 4 L of air exiting the cuvette was sampled in a multibed stainless steel cartridge (8.88 × 0.65 cm, Supelco, Bellefonte, PA, USA) filled with Carbopack adsorbents (C 20/40 mesh, C 40/60 mesh, and X 20/40 mesh). The chamber air was sampled at a flow rate of 200 mL min^{-1} for 20 min using a 1003-SKC constant flow sampling pump (SKC Inc., Houston, TX, USA) at room temperature. Background air samples were taken before and after the measurements using the same system without the leaves enclosed in the cuvette. Using the same system, CO_2 and H_2O concentrations have been measured. The

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rates of net assimilation (A) and stomatal conductance to water vapour (g_s) were calculated per unit enclosed plant leaf area according to von Caemmerer and Farquhar (1981).

Essential oils and volatile organic compounds analysis

For both essential oils and VOC analysis, a Shimadzu QP2010 Plus gas chromatograph coupled with quadrupole mass spectrometer (GC–MS) (Kyoto, Japan) was used. The conditions for essential oils analysis were as follows: injector temperature was 215°C, initial oven temperature at 40°C was held for 1 min; ramped at 5°C min^{-1} up to 200°C, held at this temperature for 1 min; ramped at 10°C min^{-1} up to 220°C and held for further 5 min. Helium (purity 99.9999 %, Elmer Messer Gaas AS, Tallinn, Estonia) was employed as carrier gas with a constant flow rate of 1 mL min^{-1}. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 30 – 400, the transfer line temperature was set at 240°C and ion-source temperature at 150°C.

For VOC analysis, an automated cartridge desorber Shimadzu TD20 (Kyoto, Japan) was used. The volatiles were analyzed according to the method described in detail in Copolovici *et al*., 2009.

The essential oils and volatile organic compounds were identified by comparing the mass spectra of individual compounds with the spectra of GC purity external standards (Sigma Aldrich, St. Louis, MO, USA) and with the spectra of NIST Library.

Statistical analysis and data handling—For transmission electron microscopy (TEM) analyses, five replicate measurements, and for essential oils and volatile organic compound three replicates with independent samples of plants were available and we report means of the replications ± SE at each treatment. The means were statistically compared with Student ANOVAs followed by post hoc Tukey's tests using ORIGIN 8 (OriginLab Corporation, Northampton, MA, USA). All statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ultrastructural analyses and photosynthetic parameters

Irradiation resulted in both qualitative and quantitative modifications in leaf anatomy. Palisade and spongy-parenchyma cells exhibit slightly wavy walls in leaves of irradiated plants, while in leaves of control plants were straight-walled (Fig. 1).

This indicates alterations in spatial arrangement of cells in leaf lamina cross-section and a moderate decrease of lamina turgidity. In fact, cell wall thickness was reduced by microwave-irradiation, and generally more strongly by the treatment with WLAN-frequency microwaves (Table 1).

Chloroplasts retained their ultrastructure and normal arrangement within cells, but chloroplasts tended to be smaller in irradiated leaves, especially in the case of WLAN frequency microwaves (Table 1). The ratio of starch grain area to chloroplast area was somewhat increased in treatment with GSM-frequency microwaves in *P. crispum* and *A. graveolens*, while it was reduced in WLAN-frequency microwave treatment in *P. crispum*

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(Table 1). Nevertheless, the differences among the treatments were relatively small, indicating moderate effects of microwave treatments on starch accumulation.

The mitochondrion length generally decreased in microwave-treated plants, especially in the case of plants irradiated with WLAN-frequency microwaves (Table 1). In addition, the number of mitochondrial cristae was also somewhat less, suggesting a certain decrease in their metabolic activity. Nuclei of most cells showed normal structure, however abundance of heterochromatin and presence of wavy contours tended to be greater in microwave-treated plants.

The anatomical modifications were qualitatively similar among species (Table 1). In all species, the treatment with WLAN-frequency microwaves resulted in greater anatomical changes than the treatment with GSM-frequency microwaves (Table 1). However, the microwave-induced changes were the strongest in *Anethum graveolens*, where all treatments differed from each other (Table 1) followed by *P. crispum* and *Apium graveolens*. In this species, chloroplast length did not differ among the treatments and mitochondrion length in GSM-frequency microwaves was not different from that in control plants (Table 1).

These data collectively demonstrate important alterations in foliage ultrastructure by microwave irradiation, and are in agreement with observations in wheat (*Triticum aestivum*) exhibiting pronounced cytogenetic changes in response to microwaves (Pavel et al., 1998; Balmori Martínez, 2003). It was shown that under the influence of low-intensity microwaves in the species of *Triticum aestivum*, as compared to the control plants different types of chromosomal aberrations appeared: delayed chromosomes, micronuclei, interchromosomal bridges, chromosomal fragments (Pavel et al., 1998). In meristem cells of *Pisum sativum* L. roots exposed to low-magnetic field were observed ultrastructural changes such as a noticeable accumulation of lipid bodies, development of a lytic compartment (vacuoles, cytosegresomes and paramural bodies), and reduction of phytoferritin in plastids. The most sensitive organelle to low-magnetic field application was mitochondria, whose size and relative volume in cells increased, matrix was electron-transparent, and cristae reduced (Belyavskaya, 2001).

Photosynthesis parameters (assimilation rates and stomata conductance to water vapor) have been affected by microwaves exposure (Figure 2). Even more, both parameters are influenced by the strength of the stress.

Overall, reduction in the size of organelles may indicate that photosynthesis and respiratory metabolism was somewhat impaired by microwave treatment (Louwerse and van der Zweerde, 1977; Lichtenthaler, 1981; Griffin et al., 2001; Terashima et al., 2011). On the other hand, reduction in cell wall thickness reduces mesophyll diffusion conductance to chloroplasts thereby potentially compensating for reduced physiological potentials and altered stomatal conductance to water vapor (Terashima et al., 2006; Tosens et al., 2012a; Tosens et al., 2012b; Tomás et al., 2013). However, reduced cell wall thickness reduces cellular resistance to low leaf water potentials (Niinemets, 2001). Thus, microwave irradiance may importantly decrease plant drought resistance.

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Changes in essential oils content in response to microwave irradiation: general patterns

Many aromatic plants have specialized structures for the storage of volatiles and the composition of essential oils in these storage structures is often complex (Letchamo et al., 1995; Manzan et al. 2003; Rajabi et al., 2013). Our study also observed complex composition of essential oils in the studied species, 10 compounds were detected in *P. crispum*, 11 compounds in *Anethum graveolens* and 7 compounds in *Apium graveolens*. In all species, monoterpenes constituted a significant component of the essential oil (Fig. 3). In addition, several specific benzenoids were also dominating components of the oil: apiol in *P. crispum*, and myristicin and dillapiole in *Anethum graveolens* (Fig. 3). Lipoxygenase pathway compounds were important constituents in essential oil in *P. crispum* and *Apium graveolens* (Fig. 3). In addition, the main volatile compounds in *Apium* graveolens are 3-hexen-1-ol, myrcene, α-ocimene, γ-terpinene (Fig. 3). These results broadly agree with past observations of essential oils produced by these species (Deng et al., 2003; Orav et al., 2003).

Multiple environmental factors have been shown to modify the content of essential oil (Langlille and MacLean, 1976; Gershenzon, 1984; Letchamo et al., 1994; Wannaz et al., 2003; Peñuelas et al., 2011). Such modifications in essential oil content have been often explained on the basis of hypotheses linking growth, and primary and secondary metabolism (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). According to these hypotheses, when sink activity (growth) rate decreases and carbon dioxide availability is in excess of that naturally occurring in the atmosphere, plants increase the rate of synthesis of secondary metabolites such as essential oils (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). In agreement with this hypothesis, water deficit has been shown to increase the yield of essential oil and affected its relative composition in *P. crispum* (Petropoulos, 2008). Analogously, enhanced salinity increased the essential oil yield for *Anethum graveolens* plants under stress (Ghassemi-Golezani et al., 2011).

In our study, microwave irradiation by GSM-frequency microwaves generally increased the essential oil contents (Fig. 3), while the effect of WLAN-frequency microwaves was less clear, varying from positive or negative for different compounds and species (Fig. 3). In a like manner, ozone stress induced two distinct pathways in *P. crispum* (Eckey-Kaltenbach et al., 1994) suggesting that different types and severity of stress can lead to qualitatively different responses. Taken together, these results are in partial agreement with several past observations indicating enhanced production of essential oils under stress and also are in agreement with the evidence of impaired photosynthetic and respiratory metabolism under microwave irradiation (Table 1). However, differently from essential oils, the anatomical modifications were significant under WLAN microwave irradiation (cf. Table 1 and Fig. 1c).

Species and microwave-frequency effects on essential oils

Although the effects were broadly similar among species, important species differences were observed in individual compound responses to GSM- and WLAN-frequency microwaves. Among the three plant species tested in these experiments, the strongest effects

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of microwave irradiation on essential oils were observed on *Anethum graveolens* plants (Fig. 3).

For individual compounds in *P. crispum*, the microwave irradiation produced by GSM generator statistically increased 3-hexen-1-ol, myrcene, α-phellandrene, β-phellandrene, myristicin and apiole contents. Compared to the reference, the strongest increase in response to GSM-frequency irradiation was observed for apiole (more than seven times greater content, Fig. 3a). The WLAN-frequency microwaves statistically increased the content of α-pinene, β-phellandrene, myristicin and apiol in this species (Fig. 3a).

In *Anethum graveolens* irradiated with GSM microwaves, increased content was observed for β-pinene, α-phellandrene and dillapiole (Fig. 3b). However, WLAN-frequency microwaves reduced α-phellandrene, myristicin and dillapiole content, whereas the greatest reduction was observed for myristicin (approximately to the level 18% of that in reference plants, Fig. 3b).

In *Apium graveolens*, both types of microwaves used in this study increased 3-hexen-1-ol content (Fig. 3c). Irradiation by WLAN-frequency microwaves reduced myrcene (19%) and α-ocimene (21%) contents (Fig. 3c).

Species-differences in environmental responses to stress factors have been demonstrated (El-Keltawi and Croteau, 1986; Mangas et al., 2006) although interspecific studies have been rare. Species ranking according to anatomical modifications was similar to the ranking based on essential oil changes (cf. Table 1 and Fig. 3). The structure of *Apium graveolens* leaves was the least affected by microwave irradiation and the effect on leaf chemistry was also the least in this species.

General patterns in volatile organic compounds emissions

Our data demonstrate that the emissions observed did reflect a mixture of both storage emission consisting of compounds present in essential oils and de novo emissions. The blend of volatiles was very complex and, in all plant species, the non-stressed plants also emitted monoterpenes and benzenoids present in essential oils, in some cases even compounds not-present in essential oils (Fig. 4). The number of compounds detected in the emissions was greater than in the essential oils, and characteristic de novo released stress volatiles were observed (Fig. 4). 16 compounds were detected in the emissions of *P. crispum*, 16 compounds in *Anethum graveolens* and 20 compounds in *Apium graveolens.*

There was evidence of similar enhancement of essential oils and emissions for several monoterpenes, especially for GSM microwave treatments in *P. crispum* and *Anethum graveolens* (cf. Figs. 3 and 4). However, in these species, emissions were more strongly enhanced under WLAN microwave treatment, which appeared to have an inhibitory effect on the content of the same terpenoids, e.g. α-pinene and β-phellandrene (cf. Figs. 3 and 4). Although there was evidence of parallel changes in contents and emissions for some volatiles in species, and for some treatments, this evidence suggests that the storage and de novo emissions cannot be fully teased apart in the current study. Nevertheless, the data

suggest that the total emissions and especially treatment differences reflect to a large degree the microwave-induced de novo synthesized plant volatiles.

Compound-class, species- and treatment-specific differences in volatile emissions

Among the de novo emissions, green leaf volatiles (GLV), also called volatiles of lipoxygenase pathway (LOX volatiles) are released in plants in response to different stresses (Copolovici and Niinemets, 2010; Copolovici et al., 2011; Copolovici et al., 2012). GLVs are formed in the hydroperoxide lyase pathway of oxylipin metabolism from free octadecanoic fatty acids and consist usually of a mixture of C6 aldehydes and ketones (Matsui, 2006). In our study, all microwave-irradiated plants emitted the following GLVs: (*E*)-2-hexenal, (*Z*)-3-hexenol, 1-hexanol, while the emissions of GLVs were very low at the level of detection limit of our device in control plants (Fig. 4).

In general, in all plant species studied, the emissions of GLV were greater for WLAN-frequency microwaves compared to GSM-frequency microwaves (Fig. 4, $P < 0.001$ for all). These results suggest greater stress in the case of WLAN microwave irradiation, and are in agreement with the more significant changes in anatomy of leaves induced by WLAN microwaves (Table 1). Stronger GLV emissions under more severe stress have been shown for water (Capitani et al., 2009), ozone (Beauchamp et al., 2005), herbivory attack (Allmann and Baldwin, 2010) and temperature (Copolovici et al., 2012) stresses.

The GLV emissions of the *P. crispum* and *Anethum graveolens* were dominated by the 1-hexanol (Fig. 4), while in *Apium graveolens* the main component was (*Z*)-3-hexenol that was also important constituent in the essential oil in this species (Figs. 3 and 4). The total GLV emission from *P. crispum* and *Anethum graveolens* was five times higher than from *Apium graveolens*. As with the essential oil content (Fig. 3), *Apium graveolens* was clearly less sensitive to the microwave fields than *P. crispum* and *Anethum graveolens*.

The monoterpenes detected in the emissions were α-pinene, β-pinene, camphene, limonene, 3-carene, *para*-cymene, β-phellandrene, (*E*)-β-ocimene, eucalyptol and bornyl acetate. In *P. crispum*, emission of α-pinene, β-pinene and β-phellandrene were dominant and enhanced by microwave irradiation, especially in the case of WLAN-frequency microwave treatment (Fig. 4a). Treatment effects on monoterpene emissions were similar for *Apium graveolens* and *Anethum graveolens*, but the main components are at some extent different (Figure 4b and 4c). Monoterpene emissions from *Anethum graveolens* were dominated by α-pinene, α-phellandrene and limonene, and these emissions were enhanced by microwave irradiation (Fig. 4b). In *Apium graveolens*, the emissions were almost four times lower than in the other species and were dominated by α-pinene, β-pinene and limonene (Fig. 4c). The emission of terpenes was inhibited by microwave irradiation similarly to the content of essential oils (Figs. 3 and 4).

Overall, these emitted monoterpenes are characteristic plant-released compounds and are not specific to stress-induced emissions (Staudt et al., 1997; Kesselmeier and Staudt, 1999; Staudt et al., 2000; Niinemets et al., 2010b). However, the emission rates of these typical monoterpenes is also often enhanced in stress conditions (Vuorinen et al., 2004; Blande et al., 2007; Heijari et al., 2008; Copolovici et al., 2011; Copolovici et al., 2012), implying that

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induced and constitutive emission are often difficult to separate. Among the characteristic induced monoterpenes (Staudt and Bertin, 1998; Hakola et al., 2001; Noe et al., 2006; Niinemets et al., 2010b), emissions of (*E*)-β-ocimene and 1,8-cineole were strongly enhanced by microwave-irradiation in *Anethum graveolens* (Fig. 4). In addition, both *P. crispum* and *Anethum graveolens*, emitted in low amounts longicyclene, a stress induced sesquiterpene, under WLAN-frequency irradiation.

CONCLUSIONS

The presented data collectively suggest that microwave irradiation constitute a stress to the plants, resulting in enhanced emissions of green leaf volatiles, up-regulation of terpenoid emissions and modification in essential oil content and foliage anatomy. Anatomical and emission traits suggested that WLAN-frequency irradiation resulted in more severe stress than GSM-frequency irradiation, but the effect of WLAN-frequency irradiation on essential oil was inhibitory. There was an agreement between anatomical and chemical traits with anatomically most resistant species *Apium graveolens* being chemically least responsive.

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Abbreviations

VOC	volatile organic compounds
GLV	green leaf volatiles
WLAN	wireless router
GSM	mobile devices
TEM	transmission electron microscopy

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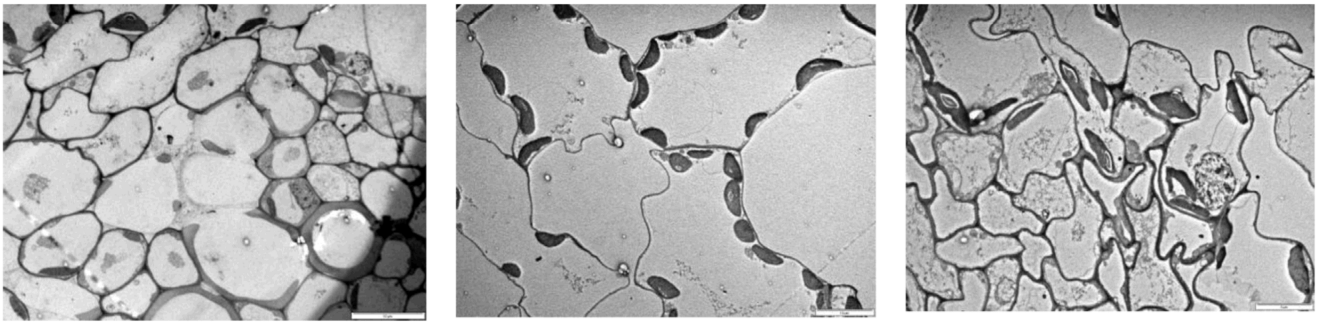

Fig. 1.

TEM images of cell walls in leaves of microwave-irradiated and control parsley: a) Control; b) GSM irradiated; c) WLAN iradiated.

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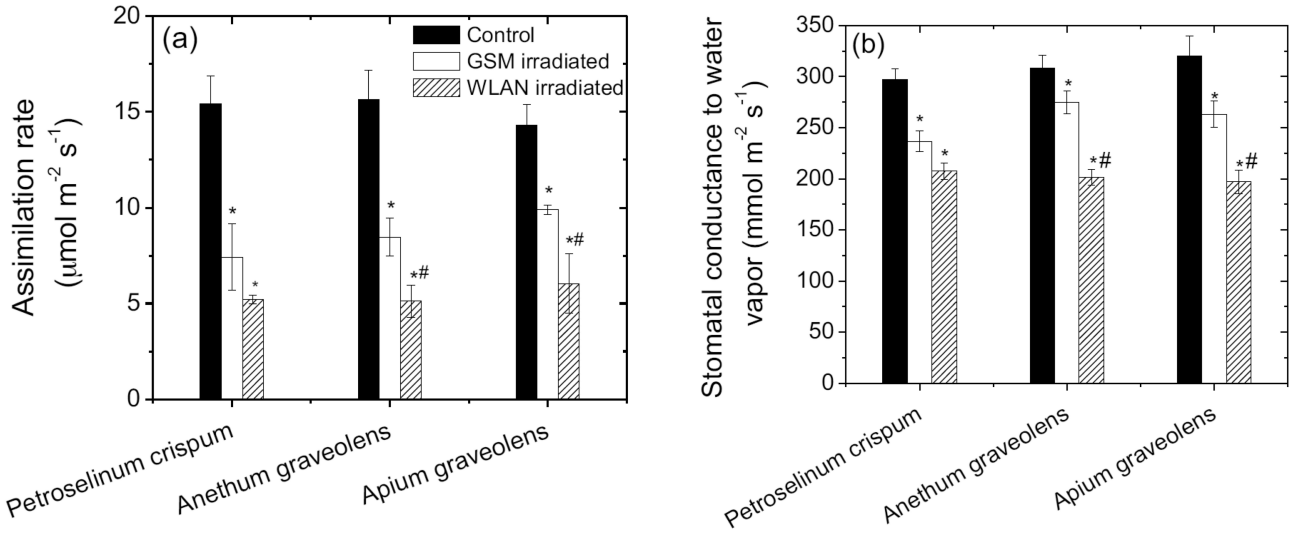

Fig. 2.

Changes in net assimilation rate (a) and stomatal conductance to water vapour (b) in 3 aromatic plants in response to microwave stress. The data are expressed per unit projected leaf area. Each data point is the mean (± SE) of 8 independent replicate experiments with a different plant.

* and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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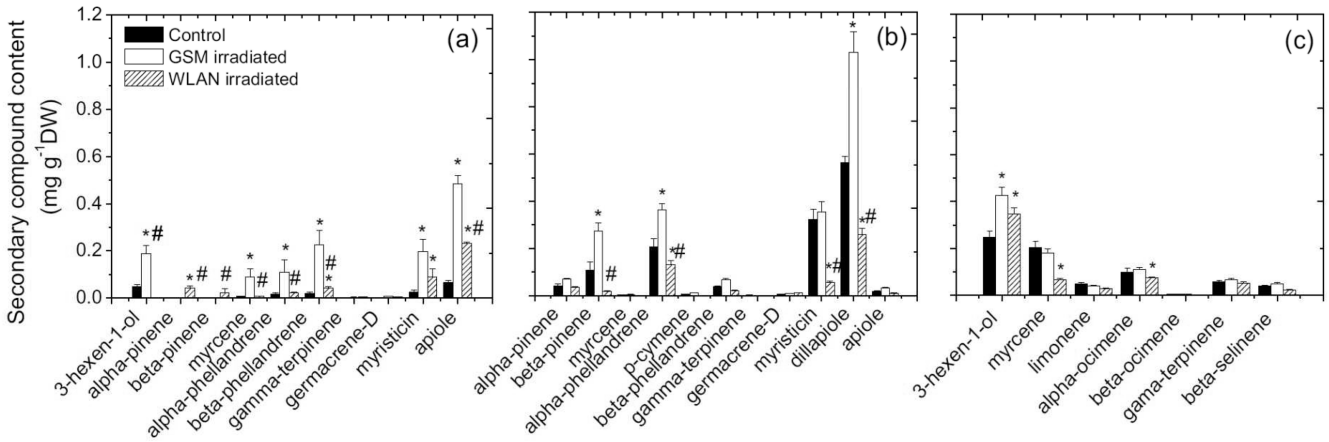

Fig. 3.

Changes in terpene content (mg g^{-1} FW) in *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) foliage in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM). Each data point is the mean (± SE) of three independent replicate experiments with a different plant. * and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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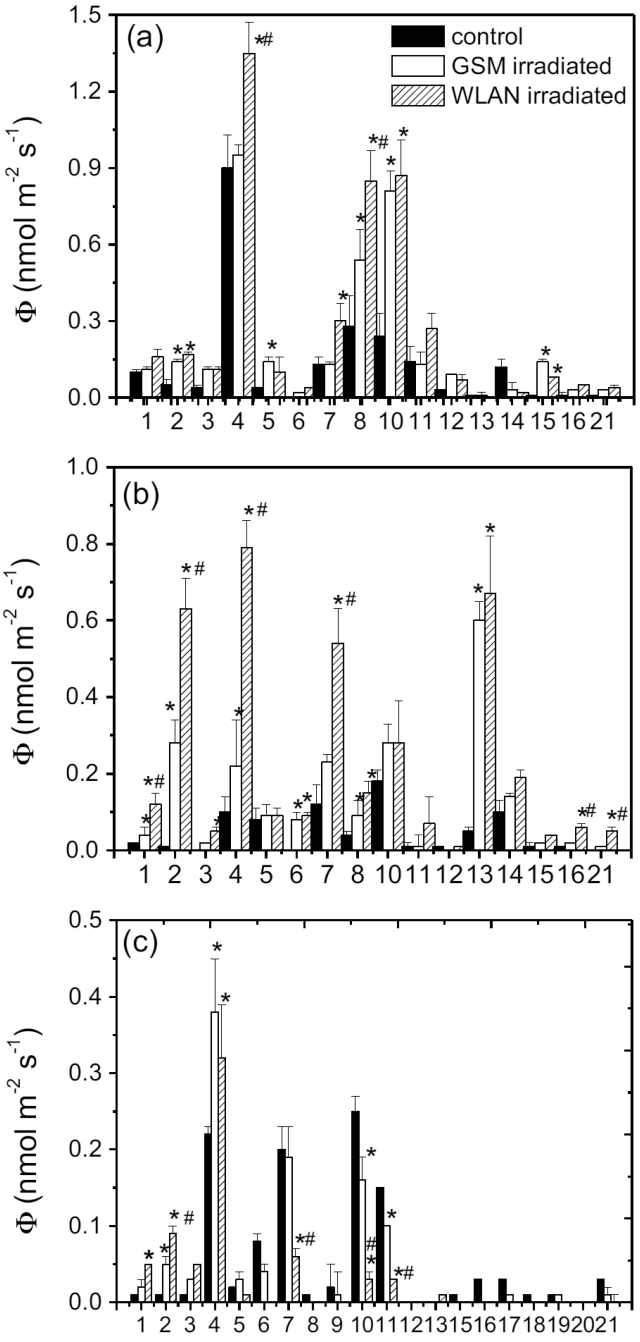

Fig. 4.

Alteration of the emission of volatile organic compounds (nmol m^{-2} s^{-1}) from foliage of *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM) (presentation of statistical differences as shown in Fig. 2). Each number corresponds to a particular volatile compound as follows: **1.** 1-hexanol; **2.** *(Z)*-3-hexen-1-ol; **3.** (*E*)-2-hexenal; **4.** α-pinene; **5.** camphene; **6.** β-myrcene; **7.** β-pinene; **8.** α-phellandrene; **9.** Δ-3-carene; **10.** D-limonene; **11.** *para*-cymene; **12.** β-phellandrene; **13.** (*E*)-

β-ocimene; **14.** 1,8-cineol; **15.** iso-bornyl acetate; **16.** longicyclene; **17.** caryophyllene oxide; **18.** α-selinene; **19.** (*Z*)-β-farnesene; **20.** α-caryophyllene; **21.** geranylacetone.

* and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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Table 1

Ultrastructural analysis of the leaves of studied plants. The average values (± SE) are replicates of six independent measurements with different plants.

Treatment	Cell wall thickness (μm)	Chloroplast length (μm)	Chloroplast area (μm^2)	Mitochondrion length (μm)	Ratio of starch grain area to chloroplast area (%)
Petroselinum crispum					
Control	0.300 ± 0.07	6.78 ± 0.12	13.31 ± 0.22	1.00 ± 0.27	8.93 ± 0.13
860 – 910 MHz	0.250 ± 0.06	6.76 ± 0.28	8.491 ± 0.06	0.90 ± 0.13	9.99 ± 0.12
2.4 - 2.5 GHz	0.200 ± 0.05	6.50 ± 0.16	7.807 ± 0.11	0.70 ± 0.05	6.01 ± 0.08
Anethum graveolens					
Control	0.187 ± 0.01	5.90 ± 0.13	8.43 ± 0.23	1.68 ± 0.13	5.21 ± 0.13
860 – 910 MHz	0.175 ± 0.01	5.20 ± 0.17	8.08 ± 0.29	1.00 ± 0.25	8.13 ± 0.08
2.4 - 2.5 GHz	0.175 ± 0.01	4.85 ± 0.31	7.04 ± 0.22	0.80 ± 0.10	0
Apium graveolens					
Control	0.160 ± 0.01	5.80 ± 0.20	7.68 ± 0.14	1.57 ± 0.08	5.34 ± 0.28
860 – 910 MHz	0.156 ± 0.01	4.33 ± 0.26	7.06 ± 0.35a	0.55 ± 0.13	0
2.4 - 2.5 GHz	0.136 ± 0.01	3.33 ± 0.26	6.68 ± 0.14	0.25 ± 0.13	0